

**International Symposium held in Vienna, 16–20 October 2000  
Organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food  
and Agriculture**



# **Nuclear techniques in integrated plant nutrient, water and soil management**



**I N T E R N A T I O N A L   A T O M I C   E N E R G Y   A G E N C Y**

PROCEEDINGS

International Symposium held in Vienna, 16–20 October 2000  
Organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture



# ***Nuclear techniques in integrated plant nutrient, water and soil management***

INTERNATIONAL ATOMIC ENERGY AGENCY

The originating Section of this publication in the IAEA was:

Soil and Water Management & Crop Nutrition Section  
International Atomic Energy Agency  
Wagramer Strasse 5  
P.O. Box 100  
A-1400 Vienna, Austria

NUCLEAR TECHNIQUES IN INTEGRATED PLANT NUTRIENT,  
WATER AND SOIL MANAGEMENT

IAEA, VIENNA, 2002

IAEA-CSP-11/P

ISSN 1563-0153

© IAEA, 2002

Printed by the IAEA in Austria

April 2002

## FOREWORD

The need to produce sufficient food of acceptable quality in the context of an ever-expanding human population has been recognized as a priority by several international conventions and agreements. Intensification, rather than expansion of agriculture into new areas, will be required if the goal of food security is to become a reality. Problems related to the sustainable production of food, fuel and fibre, both in low input and in high input agricultural systems, are now widely recognized. The over-exploitation of the natural resource base has led to serious declines in soil fertility through loss of organic matter, nutrient mining, and soil erosion. The overuse of external inputs of water and manufactured fertilizers has resulted in salinization and pollution of ground and surface waters. Nuclear science has a crucial role to play in supporting research and development of sustainable farming systems.

An FAO/IAEA International Symposium on Nuclear Techniques in Integrated Plant Nutrient, Water and Soil Management, held in Vienna from 16 to 20 October 2000, was attended by 117 participants representing forty-three countries and five organizations. The purpose was to provide an international forum for a comprehensive review of the state of the art and recent advances made in this specific field, as well as a basis for delineating further research and development needs. The participation of soil, crop and environmental scientists, as well as isotope specialists, ensured an exchange of information and views on recent advances in interdisciplinary and multidisciplinary approaches to addressing problems in sustainable land management.

The symposium was organized around seven themes, each represented by a technical session introduced by a keynote speaker.

- Evaluation and management of natural and manufactured nutrient sources
- Soil organic matter dynamics and nutrient cycling
- Soil water management and conservation
- Plant tolerance to environmental stress
- Environmental and pollution studies
- Assessment of soil erosion and sedimentation
- Recent advances in isotope analytical methodologies and related instrumentation

The symposium not only demonstrated the dynamic and evolving role of isotopes in monitoring and improving the nutrient and water status of soils, and thereby the sustainability of natural resource use for crop production, but served to increase awareness among the international scientific and development communities of recent advances in methodologies and approaches. In particular, attention was drawn to the substantial opportunities now available for improving the sensitivity and precision of stable and radioactive isotope determination through better instrumentation, and to new multiple labelling approaches to follow the cycling of two or more nutrients simultaneously and which illustrated clearly the interdependence between nutrient and carbon fluxes.

The IAEA officer responsible for this publication was P. Chalk of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. A. Eaglesham edited these proceedings for publication.



## *EDITORIAL NOTE*

*This publication has been prepared from the original material as submitted by the authors. The views expressed do not necessarily reflect those of the IAEA, the governments of the nominating Member States or the nominating organizations.*

*The use of particular designations of countries or territories does not imply any judgement by the publisher, the IAEA, as to the legal status of such countries or territories, of their authorities and institutions or of the delimitation of their boundaries.*

*The mention of names of specific companies or products (whether or not indicated as registered) does not imply any intention to infringe proprietary rights, nor should it be construed as an endorsement or recommendation on the part of the IAEA.*

*The authors are responsible for having obtained the necessary permission for the IAEA to reproduce, translate or use material from sources already protected by copyrights.*

## CONTENTS

### OPENING SESSION

Opening Statements.....	1
<i>W. Burkart, W.E.H. Blum, C. Hera, P.M. Chalk</i>	

### EVALUATION AND MANAGEMENT OF NATURAL AND MANUFACTURED NUTRIENT SOURCES (Session 1)

Innovations in isotope techniques to enhance the evaluation and management of nutrient sources (IAEA-SM-363/83).....	9
<i>G. Blair, R. Till</i>	
Dissimilatory nitrate reduction to ammonium and responsible microflora in two contrasting paddy-rice soils (IAEA-SM-363/1) .....	17
<i>S.X. Yin, D. Chen, L. Chen, R. Edis</i>	
Green-manure ( <i>Gliricidia sepium</i> ) effects on growth and yield of coffee and quantification of nitrogen recovery using $^{15}\text{N}$ dilution (IAEA-SM-363/7) .....	26
<i>W.D.L. Gunaratne, A.P. Heenkenda</i>	
Nitrogen recovery by maize from a combination of mucuna residues and inorganic N in the derived savannah of southern Benin using $^{15}\text{N}$ (IAEA-SM-363/4) .....	32
<i>P. Houngnandan, N. Sanginga, B. Vanlauwe, O. Van Cleemput</i>	
Evaluation of agronomic effects of rock phosphate using radioisotope techniques (IAEA-SM-363/3) .....	39
<i>I. Bogdevitch, S. Tarasiuk, Y. Putyatin, G. Pirogovskaya, V. Soroka, F. Zapata</i>	
Using $^{32}\text{P}$ methodology to elucidate root distribution and competition for nutrients in intercropped plant communities (IAEA-SM-363/5).....	47
<i>H. Hauggaard-Nielsen, P. Ambus, E.S. Jensen</i>	
Transfer of phosphorus in soil-plant and soil-solution systems using isotopic labeling and dilution methods (IAEA-SM-363/6) .....	49
<i>C. Morel, A. Schneider, D. Plenet, J.C. Fardeau</i>	
Isotopic studies on the fertilizer value of sewage sludge for increased crop yield and to preserve the environment (IAEA-SM-363/2) .....	57
<i>S. Ahmed, S.M. Rahman, M.B. Hossain</i>	
Nitrogen and phosphorus fertilization of rice ( <i>Oryza sativa</i> L.) grown in an acid-sulphate soil of the Mekong delta, Viet Nam (IAEA-SM-363/9).....	64
<i>Thu Tra. Luong, Le Dac Lieu, Van Tam Hoang, An Cuong Pham</i>	

### SOIL ORGANIC MATTER DYNAMICS AND NUTRIENT CYCLING (Session 2)

C and N cycling in soil: Advances in the application of $^{13}\text{C}$ and $^{15}\text{N}$ techniques (IAEA-SM-363/88) .....	71
<i>S. Recous, B. Mary, B. Nicolardot</i>	
Effect of tillage system on gross mineralization rate of a Typic Argiudoll soil (Argentina) (IAEA-SM-363/10) .....	80
<i>A. Pazos, C. Videla, H. Echeverria, G. Studdert, P.C. Trivelin</i>	
Use of natural abundance measurements of $^{15}\text{N}$ in residual nitrate assessing denitrification in buffer strips (IAEA-SM-363/11) .....	85
<i>K. Dhondt, P. Boeckx, O. Van Cleemput, G. Hofman</i>	
Soybean benefit to a subsequent wheat crop in a cropping system under zero tillage (IAEA-SM-363/12).....	87
<i>B.J.R. Alves, L. Zotarelli, R.M. Boddey, S. Urquiaga</i>	
A $^{15}\text{N}$ pool-dilution approach to measure gross N-transformations following applications of domestic sludge and compost to a field soil (IAEA-SM-363/13) .....	94
<i>P. Ambus, L.K. Kure, E.S. Jensen</i>	

Microbial assimilation of organic N from decomposing crop residues determined using $^{15}\text{N}$ pool dilution and mirror imaging (IAEA-SM-363/14).....	96
<i>E.S. Jensen</i>	
Effects of tillage and cropping on the dynamics of soil organic matter (IAEA-SM-363/15) .....	101
<i>K.M. Manjaiah, R.P. Voroney</i>	
Below ground nitrogen in fababean and chickpea (IAEA-SM-363/16).....	113
<i>D.F. Khan, D. Chen, D.F. Herridge, G.D. Schwenke, M.B. Peoples</i>	
Seasonal variability of soil $\text{CO}_2$ flux and its isotopic composition ( $\delta^{13}\text{C}$ , $\delta^{14}\text{C}$ , $\delta^{18}\text{O}$ ) (IAEA-SM-363/17) .....	119
<i>Z. Gorczyca, T. Kuc, K. Rozanski</i>	
Can the $^{13}\text{C}$ natural abundance technique be applied to quantify the soil organic matter in crop rotations with mixed $\text{C}_3$ and $\text{C}_4$ plants? (IAEA-SM-363/18) .....	127
<i>S.D. Wanniarachchi, R.P. Voroney</i>	
Production of labelled plant materials to trace the fate of residue-derived carbon, nitrogen, and sulphur (IAEA-SM-363/19).....	133
<i>P. Basilio-Sanchez, G. Blair, R. Till, M. Faint</i>	
Nitrogen dynamics and balance in a lowland rice cropping system (IAEA-SM-363/20) .....	141
<i>S. Phongpan, A.R. Mosier</i>	

### **SOIL AND WATER MANAGEMENT AND CONSERVATION (Session 3)**

Exploits and endeavours in soil water management and conservation using nuclear techniques (IAEA-SM-363/84).....	151
<i>S.R. Evett</i>	
Water use of cereal-canola-lucerne rotations in southeastern Australia (IAEA-SM-363/21).....	178
<i>C.J. Smith, W.J. Bond, K. Verburg, F.X. Dunin</i>	
Fate of nitrogen from mineral fertilizer and liquid manure over a two-year crop rotation (IAEA-SM-363/22) .....	185
<i>P. Cepuder</i>	
Effect of nutrient and soil management on the efficiency of nitrogen and water use in rainfed wheat in China (IAEA-SM-363/23).....	196
<i>Guixin Cai, Tinghui Dang, Shengli Guo, Mingde Hao</i>	
Fate of fertilizer nitrogen applied to wheat in the Mediterranean region (IAEA-SM-363/79) .....	203
<i>C. Kirda, R. Derici</i>	
Nitrogen balance in irrigated potatoes in sandy-textured soils (IAEA-SM-363/25).....	209
<i>M.B. Halitligil, A. Akin, A. Ilbeyi</i>	
Overview of the IAEA programme on fertigation studies in the Mediterranean region (IAEA-SM-363/26) .....	217
<i>P. Moutonnet, L.K. Heng</i>	

### **PLANT TOLERANCE TO ENVIRONMENTAL STRESS (Session 4)**

Genetic diversity of plants and its exploitation for stress environments (IAEA-SM-363/90) .....	237
<i>S.H.M. Naqvi, R. Bilal</i>	
Are plants in the Chinese Taklamakan Desert water limited? A stable-isotope approach (IAEA-SM-363/27).....	239
<i>S.K. Arndt, A. Foetzki, F.M. Thomas, M. Popp</i>	
Carbon isotope signatures of leaf carbon fractions: A new approach for studying short term water deficits of plants (IAEA-SM-363/28).....	243
<i>S. Heintel, W. Wanek, A. Richter</i>	
Impact of soil moisture on nodulation, biological nitrogen fixation and yields of selected tropical legumes (IAEA-SM-363/29) .....	245
<i>U.R. Sangakkara, A.D.A. Ratnayake, K.S. Kumarasinghe</i>	

## ENVIRONMENTAL AND POLLUTION STUDIES (Session 5)

Use of nuclear techniques in environmental and pollution studies (IAEA-SM-363/87).....	253
<i>M.H. Gerzabek, G. Haberhauer, A. Krenn, T. Shinonaga</i>	
Radioecological assessment of environmental pollution by naturally occurring radionuclides in the region of an ex-uranium mine and plant (IAEA-SM-363/30) .....	268
<i>R.M. Kamenova-Totzeva, N.I. Shopov, R.B. Karaivanova</i>	
Iodine-131 uptake and transfer from soil to rice following factitious contaminations (IAEA-SM-363/32) .....	272
<i>J. Balamurugan, A.R. Rajan</i>	

## ASSESSMENT OF SOIL EROSION AND SEDIMENTATION (Session 6)

Recent advances in the use of environmental radionuclides in soil erosion investigations (IAEA-SM-363/89).....	279
<i>D.E. Walling</i>	
Comparison of <sup>137</sup> Cs fallout redistribution analysis and conventional erosion prediction models (WEPP, USLE) (IAEA-SM-363/33) .....	302
<i>G. Sparovek, O.O.S. Bacchi, S.B.L. Ranieri, E. Schnug, I.C. De-Maria</i>	
Use of the <sup>137</sup> Cs technique in soil-erosion investigations: A case study in the Zitouna Basin in the north of Morocco (IAEA-SM-363/34).....	308
<i>M. Benmansour, M. Ibn Majah, H. Marah, T. Marfak, D.E. Walling</i>	
Variability of <sup>137</sup> Cs inventories in undisturbed soils across the territory of Viet Nam (IAEA-SM-363/82) .....	316
<i>P.D. Hien, H.T. Hiep, N.H. Quang, N.Q. Huy, N.T. Binh, P.S. Hai, N.Q. Long, V.T. Bac</i>	

## RECENT ADVANCEMENTS IN ISOTOPE ANALYTICAL METHODOLOGIES AND RELATED INSTRUMENTATION (Session 7)

Advances and future trends in radioisotope analysis and applications (IAEA-SM-363/85).....	325
<i>M.F. L'Annunziata</i>	
Trends in stable isotopic analyses and applications (IAEA-SM-363/86).....	355
<i>J. Oesselmann, A. Hilker, C.B. Douthitt</i>	
Characterization and dynamics of particulate organic matter in a restored river floodplain system (IAEA-SM-363/36).....	365
<i>F. Aspörsberger, F. Huber, S. Kargl, B. Scharinger, P. Peduzzi, T. Hein</i>	
Application of a simple cleanup technique for O and N isotope analysis of nitrate in natural water samples (IAEA-SM-363/37) .....	372
<i>G. Haberhauer, K. Blochberger, M.H. Gerzabek</i>	
Continuous flow pyrolysis techniques for the isotopic measurements of oxygen- deuterium in waters, organic and inorganic compounds (IAEA-SM-363/97).....	377
<i>J. Morrison, H. Hertle</i>	
Determination of iodine in cereal grains cultivated in Austria and standard reference materials by neutron activation analysis (IAEA-SM-363/38) .....	379
<i>T. Shinonaga, M.H. Gerzabek, K. Mück, J. Casta</i>	
Measuring isotopic signatures in water-soluble organic carbon (IAEA-SM-363/39).....	384
<i>R. Hood, L. Mayr, K. McTiernan</i>	

## POSTER SESSION

Use of nuclear techniques to evaluate management practices for common bean: A new P fertilizer based on partially solubilized rock phosphate (IAEA-SM-363/54P) .....	393
<i>A. García, G. Hernández, A. Nuviola, D. Montange, J.J. Drevon</i>	
Evaluation of the effects of irradiated sewage sludge on plant growth (IAEA-SM-363/40P) .....	396
<i>S. Lopez, J.P. Bonetto, N. Barbaro</i>	

Additional advantages of irradiation of sewage sludge for agriculture use (IAEA-SM-363/41P).....	398
<i>C. Magnavacca, J.G. Graiño</i>	
Gross mineralization from plant residues using cross-labelling (IAEA-SM-363/42P).....	400
<i>C.C. Videla, R.C. Hood</i>	
Diviner 2000®: A new portable hand-held depth-recognizing soil-moisture capacitance probe (IAEA-SM-363/43P).....	402
<i>P. Buss, A. Fares, M. Dalton</i>	
Effects of perennial pasture species on clover N fixation assessed by the <sup>15</sup> N-dilution method (IAEA-SM-363/44P).....	404
<i>J. Evans, B.S. Dear, G. Sandral, M.B. Peoples</i>	
Importance of drought stress and nitrogen fixation in the desert legume <i>Alhagi sparsifolia</i> — results from <sup>13</sup> C and <sup>15</sup> N natural-abundance studies in the field (IAEA-SM-363/45P).....	406
<i>S.K. Arndt, A. Kahmen, C. Arampatsis, M. Popp</i>	
A new approach to nitrogen nutrition of robusta coffee in Côte d'Ivoire (IAEA-SM-363/52P).....	408
<i>K. Ngoran, N.J. Nguessan, A. Konan, G. Yoro</i>	
Nitrogen recycling in a potato-maize-potato sequence (IAEA-SM-363/53P).....	410
<i>G. Dueñas, O. Muñiz, T. Lopez, F. Zapata</i>	
Modification of phosphate-ion transfer in a cropped Colombian oxisol with P fertilization (IAEA-SM-363/56P).....	412
<i>C. Morel, P.G. Sabo, D. Friesen</i>	
Determination of uranium uptake by plants by means of inductively coupled plasma mass spectrometry (IAEA-SM-363/57P).....	414
<i>J. Fleckenstein, E. Schnug, M.C. Meyer, T. McLendon, D. Price</i>	
Utilization of nitrogen by two rice varieties at various NPK levels (IAEA-SM-363/58P).....	416
<i>M.B. Oncsik, F. Kőrösi</i>	
Evaluation and management of micronutrients for optimizing rice productivity in valley soils (IAEA-SM-363/59P).....	418
<i>P. Nongkynrih, A.K. Singh, G. Dkhar</i>	
Effect of agricultural countermeasures on the transfer factor of caesium-137 in onion ( <i>Allium cepa</i> L.) (IAEA-SM-363/60P).....	420
<i>P.V. Jegadeeswari, M. Shanmugam, A.R. Rajan</i>	
Nitrogen contribution of green manure to corn on an ultisol using <sup>15</sup> N methodology (IAEA-SM-363/62P).....	422
<i>Nurhajati Hakim, M. Helal</i>	
The use of <sup>32</sup> P and <sup>15</sup> N to estimate fertilizer efficiency in oil palm (IAEA-SM-363/63P).....	424
<i>Elsie L. Sisworo, Widjang H. Sisworo, Havid Rasjid, Syamsul. Rizal, Z. Poeloengan, Kusnu Martoyo</i>	
The use of nuclear techniques for optimizing fertilizer application to irrigated wheat (IAEA-SM-363/95P).....	427
<i>L.K. Heng, P. Moutonnet</i>	
Aspects of fertilizer-N recovery and balance in fertigated potato in central Bekaa, Lebanon (IAEA-SM-363/65P).....	430
<i>T. Darwish, T. Atallah, S. Hajhasan, A. Chranek</i>	
Recycling of crop-residue N for sustainable production in a maize-groundnut rotation system (IAEA-SM-363/66P).....	432
<i>Rosenani Abu Bakar, Siti Zauyah Darus, Mubarak Abdelrahman Abdalla</i>	
Application of sewage sludge for corn cultivation on a tropical acid soil (IAEA-SM-363/67P).....	434
<i>Rosenani Abu Bakar, Che Fauziah Ishak</i>	
The potential of fresh leaves to improve acid-soil infertility (IAEA-SM-363/68P).....	436
<i>Zaharah A. Rahman, W.A.K. Wan Rashidah</i>	
Use of <sup>15</sup> N and the neutron probe in evaluating soil organic matter turnover and water management in wheat (IAEA-SM-363/70P).....	438
<i>M. Ismaili, A. Ichir</i>	
Effects of N and P and their interactive effects with rice genotypes on N <sub>2</sub> fixation (IAEA-SM-363/71P).....	440
<i>R.K. Shrestha, J.K. Ladha</i>	

Efficiency of use of fertilizer nitrogen by grapevine cultivated on sands as influenced by time of application (IAEA-SM-363/72P) .....	442
<i>G.E. Suteu, A. Serdinescu, M. Tircomnicu</i>	
Organic matter affects retention of applied fertilizer nitrogen (IAEA-SM-363/74P) .....	445
<i>U.R. Sangakkara, C.S. Kandapola</i>	
Use of $^{14}\text{C}$ to assess the age of humic substances (IAEA-SM-363/73P).....	448
<i>M.A. Anisimova, O.A. Chichagova, E. Schnug</i>	
Movement of carbon to roots of food legumes as affected by soil moisture and fertilizer potassium (IAEA-SM-363/75P).....	450
<i>U.R. Sangakkara, M. Frehner, J. Nosberger</i>	
Assessment of phosphorus availability in long term field experiments testing P-input regimes (IAEA-SM-363/76P) .....	452
<i>A. Gallet, S. Sinaj, E. Frossard, R. Flisch</i>	
Nitrogen fixation by vegetable and green-manure legumes as influenced by intercropping with maize (IAEA-SM-363/77P) .....	454
<i>M.K. Schneider, W. Richner, P. Stamp, U.R. Sangakkara</i>	
Drip fertigation for improvement of cotton yield, nitrogen recovery and water use efficiency (IAEA-SM-363/78P) .....	456
<i>M. Janat</i>	
Effects of phosphorus nutrition on growth and radiophosphorus uptake in <i>Sorghum bicolor</i> genotypes (IAEA-SM-363/81P) .....	458
<i>R. Camacho, E. Malavolta</i>	
Use of labelled plant residues to study the impact of residue incorporation on soil carbon and aggregation (IAEA-SM-363/91P) .....	460
<i>N. Blair, A.R. Till, R.D. Faulkner, K.E. Prince</i>	
Microcosms for evaluation of degradation of $^{14}\text{C}$ xenobiotics in soil (IAEA-SM-363/92P) .....	462
<i>C. in der Wiesche, F. Zadrazil, R. Martens</i>	
The use of carbon-isotope discrimination to screen wheat cultivars for tolerance of salinity and drought (IAEA-SM-363/93P) .....	464
<i>R. Shaheen, R.C. Hood</i>	
Labelling of sewage sludge with $^{13}\text{C}$ and $^{15}\text{N}$ isotopes (IAEA-SM-363/94P).....	466
<i>H. Kirchmann, Y. Cohen</i>	
Nitrogen transfer from legumes to non-legumes (IAEA-SM-363/96P).....	468
<i>G. Hardarson, M. Aigner</i>	
Change of $^{15}\text{N}$ natural abundance ( $\delta^{15}\text{N}$ ) in a forest soil receiving elevated N deposition (IAEA-SM-363/98P) .....	470
<i>H. Vervaeet, P. Boeckx, O. Van Cleemput, G. Hofman</i>	
Closing statement and synthesis.....	473
<i>P.M. Chalk</i>	
Symposium Officials.....	477
List of Participants.....	479





## **OPENING SESSION**



## OPENING STATEMENTS

**W. Burkart**

Deputy Director General  
Department of Nuclear Sciences and Applications  
International Atomic Energy Agency  
Vienna

On behalf of the Directors General of the International Atomic Energy Agency and the Food and Agriculture Organization of the United Nations, I have great pleasure in welcoming you to this Symposium on Nuclear Techniques in Integrated Plant Nutrient, Water and Soil Management.

At the outset, let me briefly highlight the mandate and objectives of the International Atomic Energy Agency, and in particular, the mission of my Department, the Department of Nuclear Sciences and Applications.

The IAEA serves as the world's intergovernmental organization for scientific and technical co-operation in the peaceful uses of nuclear technology, safety and verification. The IAEA was established as an autonomous organization of the United Nations in 1957, with a statutory mandate "to accelerate and enlarge the contribution of atomic energy to peace, health and prosperity throughout the world," as well as to improve nuclear safety and safeguard the non-proliferation of nuclear weapons. Thus, the IAEA assists its 131 Member States in the use of nuclear technology, promotes radiological and nuclear safety, and verifies, to the extent possible, that nuclear materials are not diverted away from legitimate peaceful uses for military purposes.

The IAEA works to foster the role of nuclear science and technology in support of sustainable human development. In the field of Nuclear Sciences and Applications, this involves both advancing knowledge through research and disseminating this knowledge to Member States through technical co-operation to tackle pressing worldwide challenges of mankind—hunger and malnutrition, disease, degradation of natural resources, and climate change.

The IAEA implements research through its Research Contract Programme. It supports and co-ordinates research through international networks of scientists from developing countries and senior advisors from developed countries. There were 159 active co-ordinated research projects at the end of 1999, with 107 (or 67%) within the Department of Nuclear Sciences and Applications in the areas of food and agriculture, human health, industry, hydrology, and the terrestrial and marine environments. Expenditure on research contracts and research co-ordination meetings in 1999 amounted to US\$7.5 million.

Where appropriate, the IAEA facilitates the transfer of nuclear technology to Member States through its Technical Co-operation Programme. Interregional, regional and national projects in food and agriculture, human health and nutrition, industry, environmental studies, and other applications are implemented in Member States. Many of these projects contribute directly or indirectly to the goals of sustainable development and protection of the environment as set out in Agenda 21 of the 1992 UN Conference on Environment and Development.

Two laboratories, located in Monaco and in Seibersdorf, Austria, support the research and technical co-operation activities. These laboratories provide scientific and analytical services to research projects and training and quality-assurance services in the area of technical co-operation.

This Symposium, which I have the privilege to open today, is convened by the Soil and Water Management & Crop Nutrition Sub-programme of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. It will provide an international forum for a comprehensive review and analysis of the application of nuclear techniques in soil, water and nutrient management. I

am sure that you will take advantage of this important gathering for exchange of information and ideas in an interdisciplinary setting. I am confident that this meeting, during which forty-eight oral contributions and some forty posters will be presented, will lead to further developments in sustainable intensification of agricultural production and conservation of the natural resource base.

In conclusion, I wish you a very successful and scientifically rewarding symposium and a pleasant stay in Vienna. I now declare the meeting open. Thank you.

**W.E.H. Blum**  
Secretary-General  
International Union of Soil Sciences  
Vienna

May I thank the organizers for inviting me to take part in this International Symposium on Nuclear Techniques in Integrated Plant Nutrient, Water and Soil Management. It is both an honour and a pleasure to convey to all of you the best greetings on behalf of the International Union of Soil Sciences (IUSS).

For those of you who are not familiar with the IUSS, I should like to inform you briefly that it is a learned society founded in Rome in 1924. About 50,000 members in 143 countries are affiliated to IUSS through their national soil science societies, or as individual members in countries that do not have a national soil science organization. The IUSS gained full membership of the International Council for Science (ICSU) in 1993, acquiring under this aegis linkages with twenty-four International Scientific Unions such as IUPAC, IUPAP, IUBS, IGU, IUGS, IUGG, IUNS, IUTOX and their respective organizations, facilitating co-operation with these disciplines. A special co-operation exists with the International Union of Radioecologists (UIR). Moreover, under the umbrella of ICSU, we are also linked with ninety-nine National Academies of Science.

Coming back to this international Symposium, I would like to make three comments. Let me start by informing you, that 30 years ago, the average life expectancy was about 46 years. Today it is about 64 years. This remarkable increase can be considered as one of the most outstanding achievements of science, not only medical science but also natural sciences such as biology, chemistry, agricultural sciences, nutritional sciences, and environmental sciences in general, including soil science. In this context, the role of soil in the functioning of ecosystems is a key issue in two respects. In a positive sense, soil influences human longevity and well-being through adequate nutrition, including clean water, clean air and the maintenance of biodiversity. However, there is a negative aspect in cases of contamination and pollution of soil and the food chain, of drinking water resources and the environment in which we live, including other biota.

During the next decades, the increase in human life expectancy will be one of the most important goals of societies, in order to enjoy acquired social and economic wealth for as long as possible. This will not be feasible without further basic and applied research, which is the key to future development.

This brings me to my second remark about the importance of nuclear techniques in the integrated management of plant nutrients, water and soil, or, in other words, the use of nuclear techniques for the sustainable management of the soil-plant-water system in order to alleviate worldwide food problems while protecting natural resources. In this context, isotopes or nuclear-based methods are very important tools to bridge the gaps between various areas of science, which are becoming increasingly specialized. The problems and solutions are complex in nature, and innovative approaches and techniques are required to integrate specializations and complexity.

My last comment refers to the IAEA itself and the joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. I should like to congratulate you on your achievements because you are proactively contributing to the improvement of food security and the protection of natural resources at a worldwide level, not only by organizing international conferences and symposia but also by active research in close co-operation with other institutions, training young scientists at the FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf, and organizing international symposia in connection with other international events such as the World Congress of Soil Science. I am grateful to you for this co-operation with IUSS, and specifically for organizing an international symposium at the 17<sup>th</sup> World Congress of Soil Science in August 2002 in Bangkok, Thailand, under the title, Towards Integrated Soil, Water and Nutrient Management in Cropping Systems: The Role of Nuclear Techniques—to which I should like to invite all of you.

I am convinced that this International Symposium will allow a comprehensive review of the present state of the art and recent advances in the use of nuclear techniques in the soil-plant-water system, and I wish the symposium every success.

**C. Hera**  
President  
International Scientific Centre of Fertilizers  
Bucharest

The FAO/IAEA International Symposium on Nuclear Techniques in Integrated Plant Nutrient, Water and Soil Management, convened in Vienna, one of the most beautiful cities in the world, represents a welcome opportunity for me to be once again officially associated with the Soil and Water Management & Crop Nutrition Section of the Joint FAO/IAEA Division, from which I retired in June 1997.

For 37 years of a career that spanned 43 years, I collaborated closely with the IAEA, conducting applied research with nuclear techniques in soil fertility, plant nutrition and fertilizer-use efficiency.

The reputation of the Soil and Water Management & Crop Nutrition sub-programme of the Joint Division was founded upon the pioneering work on fertilizer-use efficiency by the first Divisional Director, Dr. M. Fried, and his colleagues H. Broeshart, H. Axman, V. Middelboe and C. Lamm. I am very fortunate, indeed privileged, to have participated in those activities from their inception in 1963.

The fundamental importance of this work continues to grow, as the use of fertilizers continues to represent one of the most important inputs in increasing and stabilizing soil fertility, crop productivity, and food security. A sustainable and productive agriculture cannot be achieved without the rational use of all possible sources of plant nutrients, using best practices as appropriate to each farming system under specific ecological, social and economic conditions.

One of the strategic objectives of the FAO/IAEA sub-programme in Soil and Water Management & Crop Nutrition is to develop and promote the adoption of integrated multidisciplinary approaches to nutrient and water management practices using nuclear techniques for sustainable intensification of cropping systems and conservation of the natural resource base. This approach calls for the active involvement of scientists from different organizations and from different countries in the world, and is supported by the International Scientific Centre of Fertilizer (CIEC).

For those who are not familiar with the CIEC, I would like to briefly inform you that it is a non-profit and non-governmental international scientific society, founded in 1933, under the French name: Centre International des Engrais Chimiques. As an association of scientists, scientific institutions, fertilizer and trade companies, agricultural consulting services and other fertilizer-minded institutions or persons, CIEC is a centre for international studies and activities in soil fertility and plant nutrition.



By organizing international meetings, particularly those concerning fertilizers as a means for improving yield, crop quality, soil fertility, and sustainability of land-use management, CIEC forms a bridge between science, agriculture services, the fertilizer industry, and practice.

It is well known that the stock of agricultural land will diminish as a consequence of desertification and urbanization, as well as soil degradation caused by erosion, nutrient mining, acidification, and salinization. It has been predicted that the arable area per person, which was 0.37 ha in 1981 and now stands at 0.22 ha, will decline to 0.13 ha by the year 2050. The necessary increase in food production on an ever-diminishing resource base will require more external inputs of plant nutrients due to their continued removal in agricultural products, accompanied by unavoidable non-productive losses by soil erosion, leaching, and gaseous emission into the atmosphere. Furthermore, there needs to be more emphasis on the recycling of urban solid wastes and wastewater that are rich in plant nutrients. The CIEC, as well as the Joint FAO/IAEA Division, together with their partners in the CGIAR and other advanced research institutes should continue to play an important role in arresting soil degradation and optimizing the use of scarce resources worldwide.

As already mentioned, the organization of international meetings is an important function of the CIEC. All participants at the present FAO/IAEA Symposium are cordially invited to take part in the 12<sup>th</sup> CIEC World Fertilizer Congress to be held in Beijing, China, August 3 to 9, 2001.

I would like to conclude this statement by expressing my wish for better future cooperation and joint activities between the CIEC and the Soil and Water Management & Crop Nutrition sub-programme of the Joint Division, and to wish this Symposium and the organizers every success for the benefit of humanity, especially in the field of food security and environment protection.

**P.M. Chalk**

Head

Soil and Water Management & Crop Nutrition Section  
Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture  
International Atomic Energy Agency  
Vienna

Permit me to add my words of welcome to those already expressed by Mr. Burkart, Deputy Director General, Department of Nuclear Sciences and Applications.

The Soil and Water Management & Crop Nutrition Sub-programme within the Joint Division is the convener of this FAO/IAEA International Symposium on Nuclear Techniques in Integrated Plant Nutrient, Water and Soil Management. Some of you may have been present at our last Symposium, held during this same week 5 years ago.

I would like to take this opportunity to briefly outline the objectives and rationale of the Sub-programme. Our strategic objective is to use nuclear-based techniques to support interdisciplinary research and technical co-operation activities aimed at the development and transfer of sustainable land-management practices to Member States. This objective is fully consistent with several strategic objectives of FAO in its strategic framework for 2000–2015, in particular:

- creating sustainable increases in the supply and availability of food and other products from the agricultural sector, and
- supporting the conservation, improvement and sustainable use of natural resources for food and agriculture.

The global agricultural framework within which the Sub-programme operates is strongly influenced by the outcomes of major international meetings and conventions. Foremost among these are:

- the UN Conference for Environment and Development (UNCED),
- the UN Convention to Combat Desertification,
- the Rome Declaration on World Food Security.

A recurrent theme is the need for improved productivity of land and the rehabilitation, conservation and sustainable management of land and water resources. Technology transfer and strengthening of human and institutional capacities to meet national and regional requirements are also stressed. At a recent meeting of the Board of Governors of the IAEA, the spokesperson for the African Group again emphasized the need to find practical solutions to the serious problems of land degradation and soil infertility in that region. However, these problems are not confined to Africa. They are global and they are common to essentially all systems and agroecological zones.

The Sub-programme is addressing issues of global significance in crop, soil, water and nutrient management through its research and technical co-operation activities. The Soil Science Unit at the Seibersdorf Laboratories provides research support, analytical services, quality assurance and fellowship training to the co-ordinated research and technical co-operation efforts. At the end of June 2000, five coordinated research projects and twenty-four technical co-operation projects were operational within the Sub-programme.

The seven themes selected for this International Symposium, each with an invited keynote speaker, illustrate the breadth of nuclear applications to study the dynamics and balance of nutrients and water in agricultural systems. Foremost among these applications is the use of isotopes as tracers, with increasing emphasis on natural variations in the abundance of stable isotopes of the lighter elements of importance in plant nutrition. I am confident that the state of the art will be revealed by the many excellent contributions we have included in the programme.

I therefore welcome you to the IAEA and to the city of Vienna, and hope that you will find the Symposium to be a valuable source of new information and ideas that will assist you in planning and implementing future activities towards the goal of global food security.

I thank you for your participation in the Symposium and for your kind attention.



**EVALUATION AND MANAGEMENT OF NATURAL AND  
MANUFACTURED NUTRIENT SOURCES**

(Session 1)



**Keynote Address****INNOVATIONS IN ISOTOPE TECHNIQUES TO ENHANCE THE EVALUATION AND MANAGEMENT OF NUTRIENT SOURCES**

G. BLAIR, R. TILL

School of Rural Science and Natural Resources, Agronomy and Soil Science,  
University of New England, Armidale, NSW, Australia**Abstract**

The increasing world population and the need to produce more food is putting increasing pressures on soil and water resources. Increasingly, studies of interactions between nutrients rather than single nutrient studies are becoming important as systems intensify and a wider range of nutrients is added. The incorporation of isotopes into these studies will greatly assist in understanding the driving forces that determine system productivity and sustainability. Studies of N dynamics have been greatly assisted by the use of the stable  $^{15}\text{N}$ , and studies of water, other nutrients, and C have also been assisted by the use of radioactive isotopes such as  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{32}\text{P}$ ,  $^{35}\text{S}$  and  $^{86}\text{Rb}$ . However, increasing restrictions on the use of radioactive substances are beginning to severely limit their availability for such studies. Stable isotopes such as  $^{13}\text{C}$ ,  $^{15}\text{N}$  and  $^{34}\text{S}$  offer the prospect of replacing radioactive isotopes, or of their being used in combination with radioactive isotopes, to minimize the perceived risks and/or to allow the tracing of two components in the system. The innovative use of isotopes in plant-nutrient studies utilizing direct labelling with stable isotopes, multiple direct labelling with stable and/or radioactive isotopes and/or utilizing natural abundance, reverse dilution, or a combination of direct labelling and reverse dilution, are presented.

**1. INTRODUCTION**

There are ever-increasing pressures for the development of sustainable agriculture. Over the next 25 years, the population of the world will grow by about 40% to over 8 billion [1]. At present, the number of people in the world who are undernourished is estimated to be between 500 million and 1 billion [2]. The index of gross per capita production compiled by the Food and Agriculture Organization of the United Nation (FAO), using the 1989–91 period as a baseline of 100, shows that thirty-eight of sixty-four developing countries dropped below 100 in 1999, with the worst decline in Swaziland. These create a significant increase in the demand for food and, consequently, feeding the rapidly growing world population has become a major agricultural development concern for the world.

High demand for food exerts pressure on the land resources in a number of ways, such as:

- pressure to increase production on land currently in use through increased yields and cropping intensity in space and time,
- pressure to expand the land in production, especially to crops, which inevitably means expansion to more marginal lands with increasing problems for animal production, and
- pressure on land through degradation of the resource and competition for non-agricultural uses [3].

Alexandratos [4] projected that from 1983/1985 to the year 2000, agricultural production in the developing world would increase by about 60%. Of this increase, 63% would come from increased yield, 15% from increased cropping intensity and 22% from a net increase in arable land. In many countries, marginal land or cropping systems are being increasingly relied upon for agricultural production. As this reliance increases, there will be a greater need to develop sustainable land-management systems to preserve the natural resource base. Integrated use of inorganic fertilizers and soil organic matter to provide a balanced source of nutrients and energy (carbon) will play a major role in solving soil and environmental problems. Thus, the development of systems that preserve the



resource base and avoid degradation of soil requires detailed understanding and monitoring of nutrient flows in the system.

Soil organic matter is considered a key factor in maintaining soil quality, therefore it is crucial in determining long-term soil fertility [5]. There is a great deal of evidence that declines in crop yields with continued production in many temperate and tropical areas are correlated with declines in organic matter levels [6,7]. Therefore, the maintenance of soil organic matter must be considered as one of the goals of sustainable land-management systems.

Soil organic matter provides a reservoir of plant nutrients and improves soil structure. In sandy soils, increased organic matter improves water retention and reduces the leaching of nutrients. In clay soils, added organic matter can improve soil porosity, reducing water runoff and erosion losses. Organic matter can protect the soil against increases in acidity and alkalinity, and also provide a better balanced supply of macro and micronutrients than is available in the most commonly used chemical fertilizers. Clearing and subsequent cropping affect the organic matter status of soils through changes in both the input of organic matter and its rate of turnover [8,9].

As a consequence of decreasing crop yields resulting from soil organic matter decline, there is increasing interest in using plant residues, animal and human excreta, and other organic materials to improve productivity of agricultural systems. Maximizing the return of crop residues is clearly important as it both reduces the removal of nutrients and returns organic matter

In the past, organic matter management has been approached via the use of crop residue return and green manuring [10]. The use of green-manure crops with high potential breakdown rates is appropriate in regions with cool spring temperatures, which slow decay rates such that nutrients, particularly N, are released from the green manure at a rate that has some relationship with crop demands. In tropical systems, where mineralization rates are potentially higher because of high soil temperatures at the beginning of the growing season [11], and potential leaching losses are greater because of higher intensity rainfall, a rapid release of nutrients from the residue is inappropriate. The rapid release of nutrients such as N, K, and S can lead to  $\text{NO}_3^-$ ,  $\text{K}^+$  and  $\text{SO}_4^{2-}$ , and the associated cations and anions, moving down the soil profile with the wetting front such that the establishing crop does not have ready access to the released nutrients. In soils of high hydraulic conductivity, such nutrients may be leached below the rooting zone of the crop. This pattern of nutrient release is clearly the opposite of that required for sustainable agriculture.

The fate of the carbon and nutrients released during organic matter decomposition is an important determinant of the short and long-term fertility of the system. The integration of fertilizers with residues requires a balance between the stimulation of organic matter breakdown by microbial activity and the demand of nutrients by the crop.

Given the substantial investments already made in fertilizer-manufacturing plants and the forward commitments to construct new plants, it is unlikely that radical new fertilizers will become available to farmers in the foreseeable future. Rather, modifications of existing fertilizers will probably emerge. These will likely be formulated such that they will deliver nutrients to crops at rates to match plant demands and minimise losses of nutrients to the environment.

Studies using crop residues and/or fertilizers have generally focused on nutrient uptake and yield responses to various combinations of inputs, with little or no measurement of nutrient cycling. To accurately assess the sustainability of the system, carbon and nutrient dynamics must be studied together. It has become apparent that management of residues and fertilizer to optimise the efficiency of nutrient use by crops can be achieved only on the basis of a detailed understanding of the processes regulating nutrient transfer and partitioning between the added materials, the soil, plants and animals. Unequivocal evidence of these processes is obtained only by using isotopic tracers. In these studies the dynamics of nutrients released from the organic matter and fertilizers have generally been

investigated by measuring the rate or degree of incorporation of applied isotopes into various components of the system. Studies of N dynamics have been greatly assisted by the use of the stable  $^{15}\text{N}$ , and studies of water, other nutrients and C have been assisted by the use of radioactive isotopes such as  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{32}\text{P}$ ,  $^{35}\text{S}$ , and  $^{86}\text{Rb}$ . However, increasing restrictions on the use of radioactive substances are beginning to severely limit their availability for such research.

Stable isotopes such as  $^{13}\text{C}$ ,  $^{15}\text{N}$  and  $^{34}\text{S}$  offer the prospect of replacing radioactive isotopes or of their being used in combination with radioactive isotopes, to minimise the perceived risks and/or to allow the tracing of two components in the system. The use of  $^{18}\text{O}$  to trace the movement of P in biological systems also offers prospects to replace radioactive P.

Increasingly, studies of interactions between nutrients, rather than single-nutrient studies are becoming important as systems intensify and a wider range of nutrients is added to systems.

## 2. INNOVATIONS

### 2.1. Direct labelling with stable isotopes

An increasing number of reports in the literature concern natural discrimination between  $^{12}\text{C}$  and  $^{13}\text{C}$ , ranging from studies of water use efficiency to organic matter turnover.

The difference in  $\delta^{13}\text{C}$  between C3 and C4 plants has been used in many studies to determine C turnover rates [12]. The different carbon assimilation pathways in C3 and C4 plants results in different ratios of  $^{12}\text{C}$  and  $^{13}\text{C}$  between these two groups of plants. The C3 plants have  $\delta^{13}\text{C}$  in the vicinity of  $-11\text{‰}$  whereas C4 plants have a  $\delta^{13}\text{C}$  in the vicinity of  $-26\text{‰}$ . Cerri and coworkers [13] used the differences in  $\delta^{13}\text{C}$  between the largely C3 Brazilian rainforest and C4 pasture or C4 sugarcane to show that when a productive pasture was established on cleared forest the soil C pool contained a higher proportion of reactive (labile) C than the forest. On the other hand, where sugarcane was grown, both the stable and reactive C pools declined.

In a recent study [14], cows were fed on diets consisting of perennial ryegrass (a C3 species) and maize (a C4 species). By measurement of total soil C in the 1–5 cm soil horizon, and in leachate, 150 days after applying dung derived from the maize to a C3 pasture, it was possible to calculate the proportion of dung C in the two components of the system (TABLE I). It was not possible to trace the fate of the C3 derived dung in the C3 pasture.

Similarly,  $\delta^{34}\text{S}$  is being used to ascertain the source of S in rainfall, and  $\delta^{34}\text{S}$  and  $\delta^{18}\text{O}$  have been successfully used to determine the source of S in river water in New Zealand [15]. By measuring the isotope ratio in the fertilizer used in the catchment and using the offset of  $\delta^{34}\text{S}$  and  $\delta^{18}\text{O}$  from the bedrock and rainfall, Robinson [15] found that about 20% of the  $\text{SO}_4$  in the Ruamahanga River was derived from the single superphosphate fertilizer used (Fig. 1).

TABLE I. USE OF DUNG PRODUCED FROM MAIZE (C-4 PLANT) FED TO CATTLE AND APPLIED TO A C-3 PASTURE TO FOLLOW THE FATE OF C [14]

Parameter	$\delta^{13}\text{C}$ (‰)	% of C-4 applied dung
Original soil	-28.31	
Dung	-15.40	
0–5 cm soil after 150 d	-27.63	12.6
Leachate after 150 d	-24.00	4.0

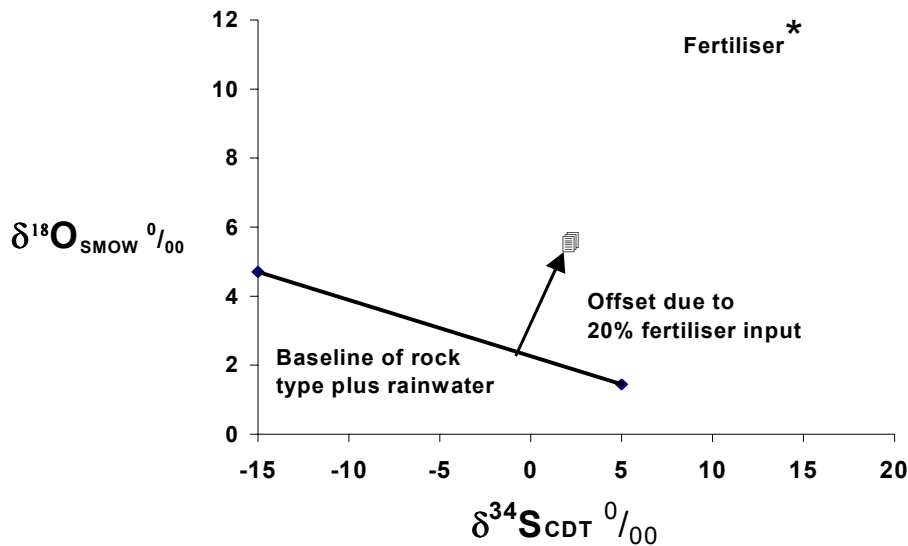


FIG. 1. Estimation of the contribution of fertilizer  $\text{SO}_4$  to riverwater S in New Zealand using stable isotopic ratios [15].

In an innovative piece of research [16] that relied on the detection of trace quantities of  $^{25}\text{Mg}$  by an ICP-MS fitted with a micro-concentric nebulizer, it was possible to establish that ectomycorrhiza is capable of enhancing the Mg supply to Norway spruce seedlings. Analyses showed that 3 to 4% of the Mg taken up by the seedlings over a 4-week period came via the mycorrhiza.

The short half-lives of P isotopes have stimulated research on the use of surrogate measures to trace P through systems.  $^{18}\text{O}$  has been used in some studies but problems exist with some of the assumptions used to interpret the results.

## 2.2. Multiple direct labelling with stable and/or radioactive isotopes and/or utilising natural abundance

Fertilizers, organic residues and animal excreta can be multi-labelled with radioactive and/or stable isotopes.  $^{15}\text{N}$ -,  $^{32}\text{P}$ -,  $^{35}\text{S}$ -, and  $^{14}\text{C}$ -labelled plants have been produced, and the fate of these elements followed in a pot experiment [17]. After 70 days, the plants had taken up a mean of 2% of the  $^{14}\text{C}$  added in the residues, 31% of the  $^{15}\text{N}$ , 40% of the  $^{32}\text{P}$ , and 41% of the  $^{35}\text{S}$ , averaged over added tops and roots.

In a pot study, C4 plant residues labelled with  $^{35}\text{S}$  and with  $\delta^{13}\text{C}$  values ranging from  $-24.91$  to  $-26.32$ ‰ were incorporated into the top 8-cm layer of a soil with a  $\delta^{13}\text{C}$  of  $-14.35$ ‰. The time course of  $^{35}\text{S}$  and  $\delta^{13}\text{C}$  values was used to show mineralization and movement of C and S through the soil layers and into the leachate [18]. The difference in  $\delta^{13}\text{C}$  between the plant residue and the soil was sufficient to be able to identify the source of C in the leachate (TABLE II).

In a study conducted in Ireland [19], differences in the isotopic ratio of C and N in clover and wheat were used to determine the feed source for earthworms. The  $\delta^{15}\text{N}$  values were sufficiently different between the clover and wheat to determine which food source was being accessed. The small difference in  $\delta^{13}\text{C}$  between the clover and wheat resulted in non-significant differences between earthworms in the two systems.

TABLE II. TRACING MOVEMENT OF  $^{35}\text{S}$ ,  $^{15}\text{N}$  AND  $\delta^{13}\text{C}$  APPLIED IN PLANT RESIDUES (% OF S, N, AND, C ADDED IN THE RESIDUES RECOVERED IN THE SOIL LAYERS AND LEACHATE) [18]

Soil layer	S		N		C	
	Flemingia	Medic	Flemingia	Medic	Flemingia	Medic
Top	5.2	11.6	79.2	56.9	7.4	2.9
Middle	1.6	4.4	1.6	5.2	1.2	4.5
Bottom	0.7	2.0	0.7	1.1	0.7	0.0
Leached	2.3	7.4	1.0	6.6	1.0	1.5

### 2.3. Reverse dilution

The use of specifically labelled materials is the best way to evaluate particular pool sizes, pathways and process rates. However, for reasons such as an inappropriate nuclide half-life or the impossibility of producing a suitably labelled material, this approach may not be possible.

An alternative approach is 'Reverse Dilution' where the system being studied is labelled with an appropriate tracer, and the effect of the particular unlabelled material is observed by measuring changes in the isotopic abundance (radioactive and/or stable) in key components of the system. An example of this technique is its use to compare the nutrient-supplying capacity of diverse materials, such as various minerals, organic matter, 'waste products,' and commercial fertilizers. This approach is versatile and powerful, but has the problem that in complex systems, such as crop and pasture production, steady-state conditions rarely, if ever, exist and any disturbance may change the relative interactions between the system components.

The requirements to be met to use reverse dilution [20] are:

- the isotope becomes distributed in the pool which supplies the plant,
- the application of the isotope does not disturb the equilibrium of the soil pools.

If neither requirement is met, it is not possible to get absolute results, but it is possible to get valid comparisons by using a range of treatment levels for each material being studied.

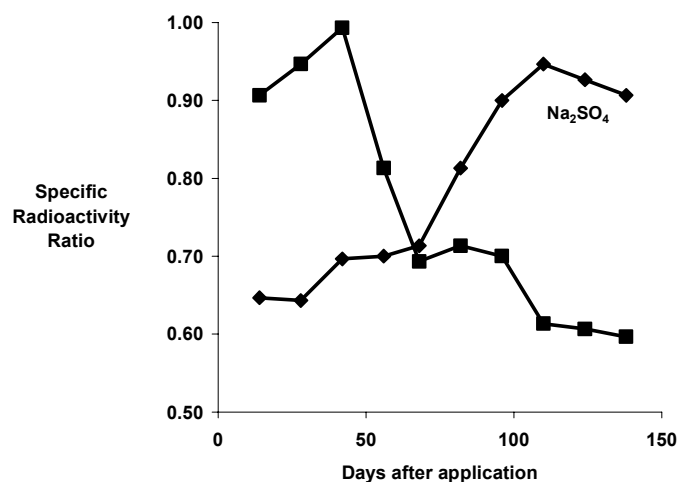


FIG. 2. Plant S uptake from  $\text{Na}_2\text{SO}_4$  and 0.1-mm diameter elemental S ( $\text{S}^0$ ) estimated by reverse dilution. (NB: the lower the SRR, the more S in the plant from the source).

In the following example of the technique, the isotopic tracer was applied to the soil in all the pots in the trial and the specific radioactivity of plants growing in each particular treatment was compared to that of plants in an untreated control. The ratio of the treated to the control (termed the Specific Radioactivity ratio, SRR) is used to estimate the nutrient contribution from the added source. A low value of SRR is obtained when the nutrient contribution is high (Fig. 2).

For example, in the system studied by Shedley et al. [20] it was shown that, irrespective of any changes within system components, the contribution to plant S from elemental S as 0.1-mm particles applied at 32 kg/ha was the same as from 0.4-mm particles applied at 240 kg/ha.

Till (unpublished) has written an excel spreadsheet that can be used to simulate changes in pool sizes and flow rates of S in a soil-plant system. By entering parameters for the pool sizes (boxes in Fig. 3) and flow rates (F-- in Fig. 3), it is possible to calculate the specific radioactivity expected in the components of the system and to compare direct labelling with reverse dilution.

## 2.4. Combination of direct labelling and reverse dilution

The rates of transfer of P from plant residues added to an acid soil into various soil-P pools, and the rates of transfer of inorganic P from the soil solution into the plant and/or other soil-P pools were studied by simultaneous use of  $^{32}\text{P}$ -labelled plant matter and  $^{33}\text{P}$ -labelled soil in the presence and absence of growing plants [21]. Equilibration of  $^{33}\text{P}$ -labelled phosphate solution added to soil reached a steady state with soil Al-P and Fe-P pools within 1 day. The Fe-P pool was much more stable than the Al-P pool since cropping did not deplete it. This non-labile pool sorbed over 30% of the  $^{33}\text{P}$  added and similar amounts of the  $^{32}\text{P}$  released from plant residues. About 50% of the  $^{32}\text{P}$  from plant residues was found in inorganic P pools 11 days after addition. This rapid release was attributed to the presence of soluble inorganic P in the residues. A further 10% was released slowly over the remainder of the experiment. Cropping only marginally slowed rates of transfer of inorganic and released residue P into non-labile pools. Cropping had no effect on the rates of release of P from crop residues.

Changes in soil-solution concentration, and the time courses of P and K uptake by cotton when subjected to short-term inundation have been followed by multi-labelling with  $^{32}\text{P}$ ,  $^{33}\text{P}$  and  $^{86}\text{Rb}$  (McLeod, unpublished). In this study,  $^{32}\text{P}$  fertilizer was applied to soil and the time course of P uptake by the plant and soil solution specific activity were monitored prior to flooding. Immediately prior to flooding,  $^{33}\text{P}$  and  $^{86}\text{Rb}$  were applied. This allowed the separation of P uptake between the equilibrated soil pools and that in the soil solution at the time of flooding. There was a dramatic reduction in P translocation into roots, stems, leaves and fruit (Fig. 4) when the plants were waterlogged and uptake of P did not recover when waterlogging ceased. Similarly  $^{86}\text{Rb}$  flux to the tops was reduced by waterlogging. These isotope data provide direct evidence that interference with P and K uptake, when cotton is irrigated at the boll-filling stage, is the primary cause of premature senescence in cotton, which occurs throughout the world.

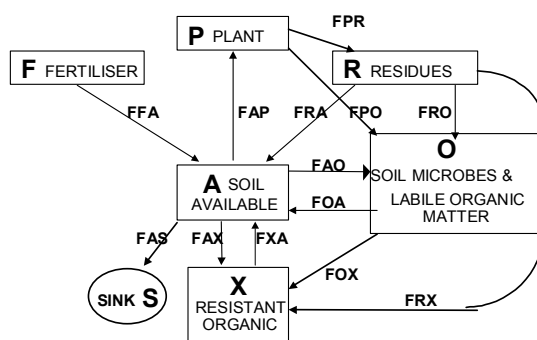


FIG. 3. Pool-size and flow-rate estimates required to simulate S cycling using reverse dilution or direct labelling using an Excel spreadsheet model (Till unpublished).

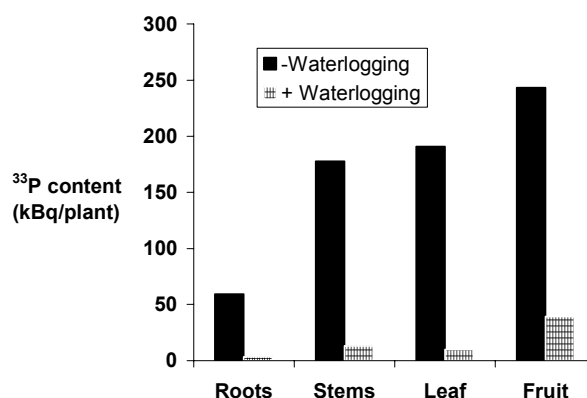


FIG. 4. Impact of waterlogging on the specific activity of P in parts of a cotton plant at boll fill when added as  $^{33}\text{P}$  at the time of inundation (McLeod, unpublished).

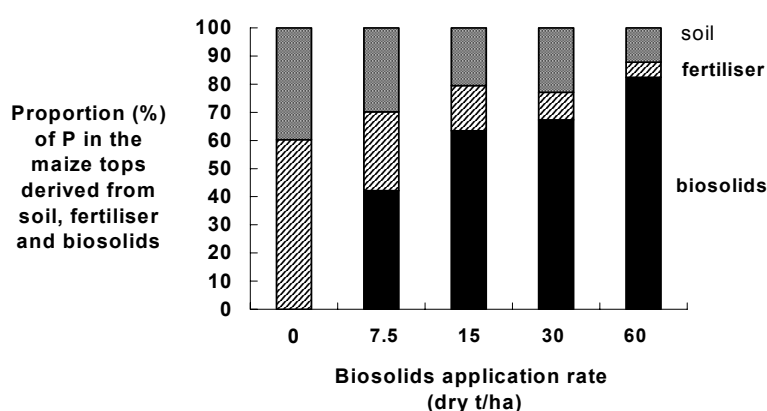


FIG. 5. Separation of P sources applied in biosolids and fertilizer using a combination of reverse dilution and direct labelling.

A similar procedure has been used to monitor the effect of Fe-treated biosolids on the availability of P from the biosolids, soil and added fertilizer [22]. A solution of orthophosphoric acid, labelled with  $^{32}\text{P}$ , was added to moist soil and left to incubate for 1 week. After this time, the biosolids from a Sydney sewage-treatment works were mixed into the soil at application rates equivalent to 0, 7.5, 15, 30 and 60 dry t/ha. Fertilizer was added as  $\text{KH}_2^{33}\text{PO}_4$  and mixed with the biosolids and soil mixtures, and maize sown. As the biosolids-application rate increased, there was an increasing proportion of P in the maize tops derived from the biosolids and less from both the soil and the fertilizer (Fig. 5).

### 3. CONCLUSIONS

As research moves into more complex areas of nutrient cycling, and pressures on nutrient turnover rates increase, it will become increasingly necessary to study multi-nutrient interactions. Examples have been presented in which multiple use of stable and/or radioactive isotopes have been employed to study nutrient-pool sizes and turnover rates, and nutrient interactions.

### REFERENCES

- [1] PINSTRUP-ANDERSON, P., PANDYA-LORCH, R., Alleviating Poverty, Intensifying Agriculture, and Effectively Managing Natural Resources, A 2020 Vision for Food, Agriculture and the Environment, Discussion Paper No. 1, International Food Policy Research Institute, Washington, DC (1994).

- [2] WORLD RESOURCES INSTITUTE World Resources 1992–93, Oxford University Press, New York, Oxford (1992).
- [3] LEFROY, R.D.B., CRASWELL, E.T., “Sustainable agriculture and natural resource management”, Nutrient Management for Sustainable Crop Production in Asia (JOHNSTON, A.E., SYERS, J.K., Eds.), CAB International, Wallingford (1998) 189–198.
- [4] ALEXANDRATOS, N., World Agriculture Toward 2000, An FAO Study, Belhaven Press, London (1988).
- [5] WALLACE, A., Soil organic matter must be restored to near original levels, Comm. Soil Sci. Plant Anal. **25** (1994) 29–35.
- [6] WOOD, A.W., The Stability and Permanence of Huli Agriculture, Department of Geography Occasional Paper No. 5 (New Series), University of Papua New Guinea and the Southern Highlands Rural Development Project, Port Moresby (1985).
- [7] SANCHEZ, P.A., et al., “Low-input systems and managed fallows for acid soils in the humid tropics”, Proc. First Regional Seminar on Soil Management under Humid Conditions in Asia (LATHAM, M., Ed.), IBSRAM, Bangkok, Thailand (1987) 297–316.
- [8] STEVENSON, F.J., Cycles of Soil; Carbon, Nitrogen, Phosphorus, Sulfur, Micronutrients, John Wiley and Sons, New York (1986).
- [9] JENKINSON, D.S., Studies on the decomposition of plant material in soil. II. Partial sterilization of soil and the soil biomass, J. Soil Sci. **17** (1966) 280–302.
- [10] MEELU, O.P., MORRIS, R.A., “Green manure management in rice-based cropping systems”, Sustainable Agriculture, Green Manure in Rice Farming, International Rice Research Institute, Philippines, 25–29 May 1987, International Rice Research Institute (1988) 209–222.
- [11] JENKINSON, D.S., AYANABA, A., Decomposition of carbon-14 labelled plant material under tropical conditions, Soil Sci. Soc. Am. J. **41** (1977) 912–915.
- [12] CERRI, C.C., et al., Application du tracage isotopique naturel en  $^{13}\text{C}$  à l’étude de la dynamique de la matière organique dans le sols, C.R Acad. Sci. Paris **300.ii (9)** (1985) 423–426 (1985).
- [13] CERRI, C.C., et al., Carbon pools and fluxes in Brazilian natural and agricultural systems and the implications for global  $\text{CO}_2$  balance, Proc. 15<sup>th</sup> World Congress Soil Sci. **5a** (1994) 399–406.
- [14] BOL, R., et al., Tracing dung-derived carbon in temperate grasslands using  $^{13}\text{C}$  natural abundance measurements, Soil Biol. Biochem. **32** (2000) 1322–1343.
- [15] ROBINSON, B.W., Discrimination of sulfur sources in pristine and polluted New Zealand river catchments using stable isotopes, Appl. Geochem. **12** (1997) 305–319.
- [16] JENTSCHKE, G., et al., The mycorrhizal fungus *Paxillus involutus* transports magnesium to Norway spruce seedlings. Evidence from stable isotope labelling, Plant Soil **220** (2000) 243–246.
- [17] TILL, A.R., et al., “Isotopic studies of the recycling of carbon, nitrogen, sulfur and phosphorus from plant material”, Cycling of Carbon, Nitrogen, Sulfur and Phosphorus in Terrestrial and Aquatic Ecosystems (FRENEY, J.R., GALBELLY, I.E., Eds.), Australian Academy of Science, Canberra (1982) 51–59.
- [18] KONBOON, Y., et al., Tracing the nitrogen, sulfur and carbon released from plant residues in a soil/plant system, Aust. J Agric. Res. **38** (2000) 699–701.
- [19] SCHMIDT, O., et al., Natural abundance of  $^{15}\text{N}$  and  $^{13}\text{C}$  in earthworms from a wheat and wheat-field, Soil Biol. Biochem. **29** (1997) 1301–1308.
- [20] SHEDLEY, C.D., et al., A radiotracer technique for studying the nutrient release from different fertilizer materials and its uptake by plants, Comm. Soil Sci. Pl. Anal. **10** (1979) 737–745.
- [21] FRIESEN, D.K., BLAIR, G.J., A dual radiotracer study of transformations of organic, inorganic and plant residue phosphorus in soil in the presence and absence of plants, Aust. J. Soil Res. **26** (1988) 355–366.
- [22] DAVIS, J., Phosphorus Availability from Iron Treated Biosolids, PhD Thesis, University of New England, Armidale, NSW, Australia (2000).

# DISSIMILATORY NITRATE REDUCTION TO AMMONIUM AND RESPONSIBLE MICROFLORA IN TWO CONTRASTING PADDY-RICE SOILS\*

S.X. YIN \*\*, D. CHEN, L. CHEN\*\*, R. EDIS  
Institute of Land and Food Resources,  
University of Melbourne, Victoria, Australia

## Abstract

The purpose of this study was to compare nitrate reduction, and the responsible microflora, in two soils contrasting in their potential for dissimilatory nitrate reduction to ammonium (DNRA). A soil from Griffith (NSW) reduced 14.5% of applied  $^{15}\text{N}$ -labelled nitrate to ammonium and organic-N under laboratory incubation without any exogenous carbon sources. A soil from Yangzhou (China) reduced only 4.7%. Addition of reducing agents (sodium thioglycollate and *L*-cysteine) enhanced the DNRA process, with most of the product of nitrate reduction being ammonium. Additions of glucose also facilitated DNRA, with most of the product being organic-N. There was a small but measurable amount of nitrous oxide production during nitrate reduction. Nitrous oxide production accounted for 0.05 to 4.2% of added nitrate by the end of experiments. Nitrous oxide tended to dissipate as the incubation proceeded in all non-glucose treatments, whereas in glucose treatments it tended to accumulate. The denitrifier population in the Griffith soil was about a tenth of the size of that in the Yangzhou soil. The DNRA population in Griffith soil, in contrast, was larger than that in Yangzhou soil. Of DNRA bacteria and denitrifiers isolated, spore-former dominated.

## 1. INTRODUCTION

Nitrate ( $\text{NO}_3^-$ ) reduction has been recognized as a major process of the N cycle in soils. Nitrate can be reduced to nitrogen gas ( $\text{N}_2\text{O} + \text{N}_2$ ) (denitrification) or to ammonium ( $\text{NH}_4^+$ ) (dissimilatory nitrate reduction to ammonium, DNRA) under anaerobic conditions, depending upon ambient conditions and the microflora present. Intensively reduced and carbon-rich environments usually favour DNRA [1–3]. Bacteria reported to be capable of DNRA include obligate (e.g. *Clostridium* spp.) and facultative anaerobes (e.g. *Enterobacter*) and aerobes (e.g. *Bacillus* spp.). The population ecology of DNRA in natural soils is, however, poorly understood.

Laboratory anoxic incubation experiments, with no added C source, have generally resulted in small fractions (usually less than 5%) of added  $\text{NO}_3^-$  being converted to  $\text{NH}_4^+$  or organic N [1, 4–5]. It was, therefore, concluded that DNRA was of little agricultural significance. In an Australian paddy soil, however, DNRA accounted for 15% of  $\text{NO}_3^-$  reduction under laboratory conditions without an exogenous carbon source [6–7], which is, to our knowledge, the highest proportion reported under similar conditions in agricultural soils. Comparative studies of this soil may help us to understand the factors affecting the DNRA process. The purpose of the present study, therefore, was to compare nitrate reduction and responsible microflora in two soils contrasting in their potential for DNRA.

## 2. MATERIALS AND METHODS

### 2.1. Soils

The Typic Pelloxerert soil collected from Griffith (34°21'S, 146°02'E), New South Wales, Australia, contains: organic C 11.2 g kg<sup>-1</sup>; total N 1.1 g kg<sup>-1</sup>, and; clay 500 g kg<sup>-1</sup>, with pH 8.4 (soil:water, 1:1). The Typic Fluvaquent, an alluvial soil derived from Yangtze River deposit, collected from Yangzhou (32°15'N, 119°40'E), China, contains: organic C 9.0 g kg<sup>-1</sup>; total N 0.8 g kg<sup>-1</sup> and clay 380 g kg<sup>-1</sup>, with pH 7.1. The two soils are herein designated as Griffith and Yangzhou, respectively. A rice crop is

\*This work was supported by the National Natural Science Foundation of China (49671049) and the Australian Research Council (ARC) (S399770).

\*\*Present address: Department of Agronomy, Agricultural College, Yangzhou University, Yangzhou 225009, China.



grown on Griffith soil once annually followed by fallow and straw mulching whereas a wheat-rice rotation is practised on the Yangzhou soil. Both soils were submerged when sampled. When not in use, the samples were kept in polyethylene bags at  $<5^{\circ}\text{C}$  (except travelling time of 1 day).

## 2.2. Soil incubation

Five-gram (oven dry basis) samples of fresh soil were placed in 210-mL bottles. Both  $^{15}\text{N}$ -labelled  $\text{KNO}_3$  (8.766  $^{15}\text{N}$  atom %) and non-labelled  $(\text{NH}_4)_2\text{SO}_4$  were added at the rate of  $100 \text{ mg N kg}^{-1}$ . Non-labelled  $(\text{NH}_4)_2\text{SO}_4$  was added so as to inhibit nitrate assimilation [8]. The bottles were sealed with rubber septa and filled with oxygen-free gas (24.5%  $\text{N}_2$  balanced with Ar) by evacuating and refilling three times. Treatments in triplicates included full combination of two levels of glucose (0 and  $1,440 \text{ mg C kg}^{-1}$ ) and reducing agents (none, 0.05% sodium thioglycollate, or 0.025% *L*-cysteine). The reducing agents were prepared as described by Kaspar and Tiedje [9]. The final volume of soil slurry was 20 mL. The bottles were incubated at  $28^{\circ}\text{C}$ . At intervals, the bottles were vigorously shaken by hand, the air pressure measured with a pressure transducer and the gas in the headspace was sampled for measurement of  $^{15}\text{N}_2 + ^{15}\text{N}_2\text{O}$ . Separate gas samples were used for measuring  $\text{N}_2\text{O}$  contents only by GC. Mineral N was extracted from the soil slurries by adding 1.49 gram of KCl (1 *M*) to the slurry, then shaking for 30 min. The samples were then centrifuged and washed twice with 10 mL of 1 *M* KCl solutions. The supernatant was collected for analyses of  $\text{NH}_4\text{-}^{15}\text{N}$ ,  $\text{NO}_3\text{-}^{15}\text{N}$  and  $\text{NO}_2\text{-}^{15}\text{N}$ . The washed residual soils were dried at  $60^{\circ}\text{C}$  and ground to pass through a 60 mesh. Residue  $^{15}\text{N}$  in the washed soils was considered to be in an organic form.

## 2.3. Microbial study

Denitrifier and nitrate-reducer populations were estimated by the Durham tube method on the basis of most probable number procedures [10]. The medium used was nitrate broth (NB, per liter: beef extract 3 g, peptone 5 g,  $\text{KNO}_3$  1 g, 5 mL of 1% bromthymol blue, pH 7.0). Soil suspensions were prepared by a ten-fold series dilution technique in sterile water plus ten glass beads and one drop of Tween-80. One-mL suspensions from each dilution were transferred to each of five tubes containing 9 mL of NB. The tubes were put into a desiccator, which was evacuated and filled with  $\text{O}_2$ -free Ar gas three times. The air pressure in the container was 0.01–0.03 bar higher than atmosphere. The tubes were then incubated at  $28^{\circ}\text{C}$  for 2 weeks. Bubbles formed in the inverted small tubes were considered to be the consequence of denitrification. Colour change of the medium from green to dark blue indicated pH increase and was considered as the consequence of  $\text{NO}_3^-$  reduction (net consumption of protons).

For bacterial isolation, a soil suspension inoculum of 0.1 mL from each dilution was spread on the surface of NB agar and NB agar plus  $10 \text{ g L}^{-1}$  glucose (NBG) in Petri dishes (70-mm diameter), which were placed in a desiccator and incubated anaerobically at  $28^{\circ}\text{C}$ . Anaerobiosis was achieved by the procedure described above. Colonies on the plates that showed appropriate numbers (usually 40–100) were all streaked on NB agar and incubated aerobically. Colonies from NB and NBG were pooled. Purification was repeatedly done by series-dilution technique. The colonies that grew on the medium anaerobically but not aerobically were considered to be obligate anaerobes; they were recorded but not studied further. A few isolates that showed sluggish growth on either medium were omitted because of difficult management.

Purified isolates were characterized for their denitrification and DNRA potentials. The medium ( $\text{NH}_4^+$ -free) for DNRA confirmation consisted of (per liter of deionized water)  $\text{Na}_2\text{HPO}_4$  3.904 g,  $\text{KH}_2\text{PO}_4$  2.721 g,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  30 mg,  $\text{KNO}_3$  1 g, glucose 10 g, 1 mL of microelement solution (per liter: EDTA 500 mg,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  200 mg,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  10 mg,  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  3 mg,  $\text{H}_2\text{BO}_7$  30 mg,  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  20 mg,  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  1 mg,  $\text{NiCl}_2 \cdot \text{H}_2\text{O}$  2 mg,  $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$  3 mg) and 2 mL of vitamin solution (biotin  $0.75 \text{ mg L}^{-1}$ , thiamine  $0.75 \text{ mg L}^{-1}$ , pantothenic acid  $0.75 \text{ mg L}^{-1}$ ) [11]. For a few isolates that did not show satisfactory growth in the medium, an autoclaved soil extract medium (consisting of  $\text{Na}_2\text{HPO}_4$  3.904 g,  $\text{KH}_2\text{PO}_4$  2.721 g,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  30 mg,  $\text{KNO}_3$  1 g, glucose 10 g, 100 mL soil extract and 900 mL of water) was used instead. Each isolate was inoculated into three tubes containing 10 mL of the required medium and incubated anaerobically at  $28^{\circ}\text{C}$  for 3 days. Non-

inoculated media were used as checks. An increase of  $\text{NH}_4^+$  in the media that was statistically different from the check was considered as the consequence of DNRA and the respective isolates as DNRA bacteria. Non-DNRA isolates were further characterized for their  $\text{NO}_3^-$  reduction by the Durham-tube method. Each non-DNRA isolate was inoculated into Durham tubes containing 10 mL of NB (without bromthymol blue) and incubated anaerobically at 28°C for 2 weeks. Presence of nitrite ( $\text{NO}_2^-$ ) was periodically checked by spot tests with sulphanilamide reagent. For the tubes that were absent of  $\text{NO}_2^-$ , presence of  $\text{NO}_3^-$  was checked by adding zinc powder and spot-testing again. By the end of incubation, the isolates that consumed all  $\text{NO}_3^-$  present (neither  $\text{NO}_2^-$  nor  $\text{NO}_3^-$  detectable) and/or showed bubble formation in the inverted small tubes, were considered as denitrifiers. Those that showed presence of  $\text{NO}_3^-$  or  $\text{NO}_2^-$  but no gas production were regarded as  $\text{NO}_2^-$  accumulators.

The denitrifiers were further reconfirmed by their linear growth with regard to the amount of  $\text{NO}_3^-$  added to NB medium. Tracking the patterns of  $\text{NO}_3^-$  reduction during growth in the  $\text{NH}_4^+$ -free medium reconfirmed DNRA isolates.

Isolates were identified at genus level by characters such as Gram stain, sporulation, motility and standard tests on the reaction to sugar (F-O test), catalase, litmus milk, citrate, gelatin, and indole.

## 2.4. Analytical methods

$\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N+ $\text{NO}_2^-$ -N in soil extracts were measured by steam-distillation procedures with MgO and MgO plus Devarda's alloy, respectively. Distillates were acidified and dried for measuring  $^{15}\text{N}$  abundances on a VG Isogas (Sira 10) mass spectrometer.  $\text{NO}_2^-$ -N was measured separately by Griess-Ilosvay colour reaction. The quantity of N and  $^{15}\text{N}$  contents in the washed residue soil was measured with a CNS Autoanalyzer (Varian NA1500) coupled with the mass spectrometer. Nitrogen-15-labelled  $\text{N}_2 + \text{N}_2\text{O}$  were determined with the same mass spectrometer. The gas inlet system for the mass spectrometer was the same as that described by Chen et al. [12]. The amount of  $\text{N}_2 + \text{N}_2\text{O}$  derived from  $^{15}\text{N}$ -labelled nitrate was calculated based on the equation derived by Mulvaney and Boast [13], using the ratio of  $^{29}\text{N}_2$ : $^{28}\text{N}_2$  and  $^{30}\text{N}_2$ : $^{28}\text{N}_2$ . The natural abundance of  $^{15}\text{N}$  in atmospheric  $\text{N}_2$  ( $0.3663 \pm 0.0004$  atom %) was taken as the time-zero value.

In bacterial characterization experiments, optical density at 530 nm was monitored as the indication of cell growth. For measurement of various N forms in media, cells were harvested by centrifuging at 15,000 rpm for 5 min at 5°C. Ammonium in the supernatant was determined manually by the indophenol blue reaction [14]. Nitrite was measured by the Griess-Ilosvay method. Nitrate was reduced to  $\text{NO}_2^-$  by passing the supernatant through a Cd column and measured colourimetrically. A detailed description is given by Daniels et al. [14].

## 3. RESULTS

### 3.1. Nitrate reduction in soils

The two soils were considerably different in their potential for DNRA. In Griffith soil, 14.5% of applied  $^{15}\text{NO}_3^-$  was reduced to  $\text{NH}_4^+$  plus organic-N after 4.5 days incubation, whereas only 4.7% was reduced in the Yangzhou soil (Table I). These data are comparable to those previously reported by Chen et al. [6] and Yin et al. [5] with Griffith and Yangzhou soils, respectively. Nitrate reduction proceeded more rapidly in Griffith soil than in Yangzhou soil, as was indicated by large amounts of  $^{15}\text{NO}_3^-$  remaining as  $\text{NO}_3^-$  plus  $\text{NO}_2^-$  in the latter after 4.5 days incubation. Prolonged incubation up to 7.5 days gave a little more  $\text{NH}_4^+$  production, but produced significantly more gaseous N. This implies that DNRA proceeded more rapidly than denitrification under the incubation conditions.

TABLE I. RECOVERY OF  $^{15}\text{N}$ -LABELLED NITRATE IN TWO SOILS UNDER ANAEROBIC INCUBATIONS WITHOUT GLUCOSE

Soil	Incubation time (h)	$(\text{NO}_3^- + \text{NO}_2^-)\text{-N}$	$\text{NH}_4^+\text{-N}$	Organic-N	$(\text{N}_2 + \text{N}_2\text{O})\text{-N}$	Total recovery (%)
		(mg N per bottle)				
Yangzhou	36	0.427 (0.007) <sup>a</sup>	0.0176 (0.0002)	0.0059 (0.0005)	0.0509 (0.0008)	101
	108	0.355 (0.006)	0.0192 (0.0002)	0.0042 (0.0007)	0.102 (0.0104)	96.9
	180	0	0.0209 (0.0003)	0.0041 (0.0007)	0.479 (0.0191)	102
Griffith	36	0.412 (0.010)	0.0364 (0.0006)	0.0089 (0.0011)	0.0498 (0.0011)	102
	108	0	0.0621 (0.0002)	0.0097 (0.0004)	0.347 (0.0520)	84.4
	180	0	0.0595 (0.0007)	0.0146 (0.0028)	0.446 (0.029)	105

<sup>a</sup>Standard deviations in parentheses

Reducing agents significantly increased DNRA (Fig. 1A). This was particularly the case for *L*-cysteine added to Griffith soil, which made the partitioning of  $\text{NO}_3^-$  to DNRA (56%) more than to denitrification (44%). Ammonium was the main product. The actual Eh of soil slurry, measured at the end of experiment, was  $-38 \pm 8$  mV for non-reducing agent (Eh1),  $-64 \pm 11$  mV for sodium thioglycollate (Eh2) and  $-171 \pm 16$  mV for *L*-cysteine (Eh3) treatments. Eh fluctuations during the experiment, although not monitored, were expected because  $\text{NO}_3^-$ , a strong poisoning agent for high Eh ( $\text{Eh}^\circ = 420$  mV at pH 7) [15], kept decreasing.

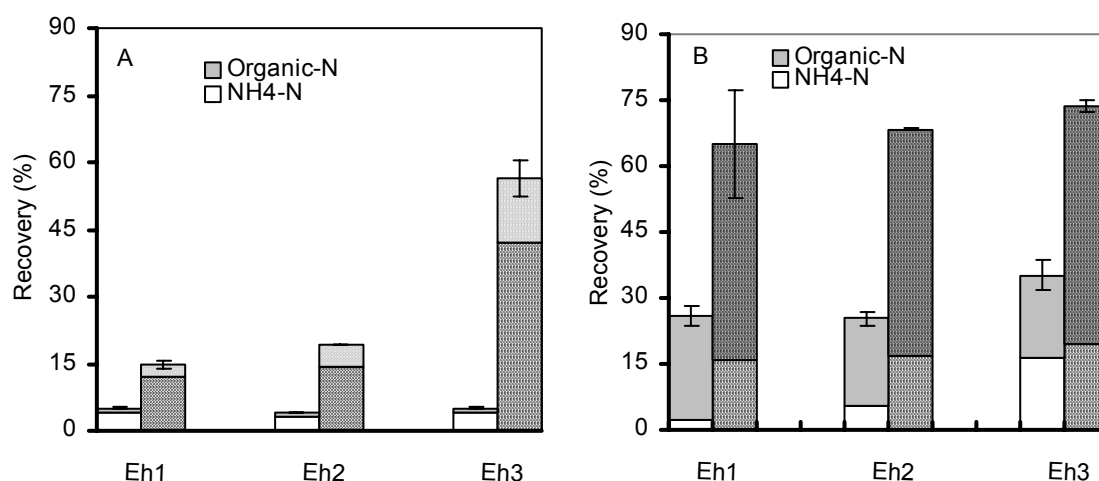


FIG. 1.  $^{15}\text{N}$ -labelled nitrate recovered as  $\text{NH}_4^+\text{-N}$  and organic-N in two soils incubated for 7.5 days under different Ehs with (B) without (A) glucose (Griffith soil: hatched; Yangzhou soil: non-hatched).

The final Eh values measured, however, indicated that different reducing status were achieved in the treatments by the reducing agents.

Glucose treatments showed again the contrasting DNRA potential capabilities of these soils (Fig. 1B).  $^{15}\text{N}$ -labelled N recovered as  $\text{NH}_4^+\text{-N}$  and organic-N accounted for 65 to 73% of  $\text{NO}_3^-$  reduction in Griffith soil and 26 to 35% in Yangzhou soil. Glucose resulted in an additional increase of DNRA (Fig. 1B) for both soil, as compared to non-glucose treatments (Fig. 1A). The additional increase was more pronounced in high-Eh treated samples than in low-Eh treated ones. In the glucose-treated samples, organic-N was the main product, in contrast to the non-glucose treatment (Fig. 1A), indicating that ammonium assimilation was stimulated by glucose. Glucose addition appeared to decrease the effect of reducing agents on DNRA because the differences among three Eh treatments for the total  $^{15}\text{N}$  recoveries in  $\text{NH}_4^+\text{-N}$  and organic-N pool were small (Fig. 1B), while significantly different in non-glucose treatments (Fig. 1A). This is also consistent with finding that the Eh differences among reducing-agent treatments (Fig. 1B) was also narrowed by glucose [ $-152\pm 17$  mV for non-reducing agent (Eh1),  $-175\pm 21$  mV for sodium thioglycollate (Eh2) and  $-208\pm 19$  mV for *L*-cysteine (Eh3) treatments, as measured at the end of experiment].

Small amounts of  $\text{N}_2\text{O}$  were produced during  $\text{NO}_3^-$  reduction (Table II).  $\text{N}_2\text{O}$  production by the end of the experiments accounted for 0.05 to 4.2% of  $\text{NO}_3^-$  added. It was interesting to note that  $\text{N}_2\text{O}$  in non-glucose treatments tended to disappear as incubation proceeded, whereas in glucose treatments it tended to accumulate. These trends were most obvious in *L*-cysteine treatments and in *L*-cysteine plus glucose treatments, respectively (Table II). This effect was more obvious in Griffith than in Yangzhou soil.

### 3.2. Nitrate-reducer populations and characterization

The denitrifier population in Griffith soil was approximately one order of magnitude smaller than that in Yangzhou soil (Fig. 2), as estimated by the most probable number method. The size of the  $\text{NO}_3^-$ -reducer populations in the two soils were similar in numbers. The denitrifier population accounted for 0.5% and 17% of  $\text{NO}_3^-$  reducers in Griffith and Yangzhou soils, respectively.

TABLE II. NITROUS OXIDE PRODUCTION AS AFFECTED BY SOIL Eh

Soil	Treatment	1.5 days incubation		4.5 days incubation		7.5 days incubation	
		$\text{N}_2\text{O-N}$ (mg) <sup>a</sup>	Recovery (%)	$\text{N}_2\text{O-N}$ (mg)	Recovery (%)	$\text{N}_2\text{O-N}$ (mg)	Recovery (%)
Yangzhou	Check	0.0005 (0.00007) <sup>b</sup>	0.103	0.0004 (0.00010)	0.076	0.0002 (0.00003)	0.049
	<i>L</i> -cysteine	0.0007 (0.00013)	0.134	0.0002 (0.00003)	0.054	0.0002 (0.00002)	0.049
Griffith	Check	0.0041 (0.00061)	0.828	0.0002 (0.00001)	0.049	0.0003 (0.00001)	0.056
	<i>L</i> -cysteine	0.0228 (0.00152)	4.595	0.0003 (0.00007)	0.052	0.0003 (0.00001)	0.052
Yangzhou	Glucose	0.0004 (0.00009)	0.072	0.0003 (0.00009)	0.062	0.0003 (0.00001)	0.062
	<i>L</i> -cysteine +glucose	0.0003 (0.00003)	0.060	0.0017 (0.00012)	0.333	0.0028 (0.00016)	0.561
Griffith	Glucose	0.0014 (0.00008)	0.274	0.0004 (0.00005)	0.072	0.0003 (0.00002)	0.053
	<i>L</i> -cysteine +glucose	0.0003 (0.00002)	0.069	0.0003 (0.00002)	0.069	0.021 (0.00001)	4.229

<sup>a</sup>mg N/bottle. <sup>b</sup>Standard deviations in parentheses.

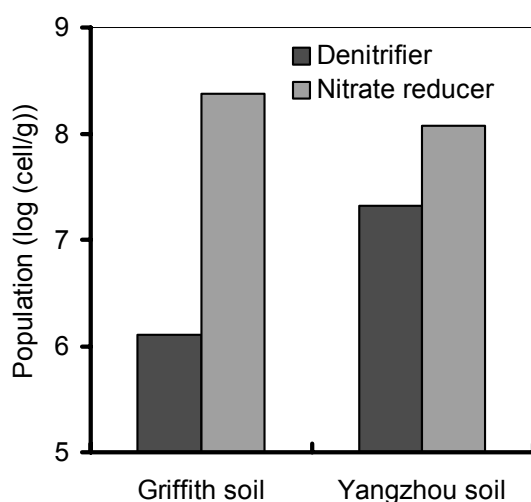


FIG. 2. Populations of denitrifiers and nitrate reducers in two soils estimated by the most probable number method.

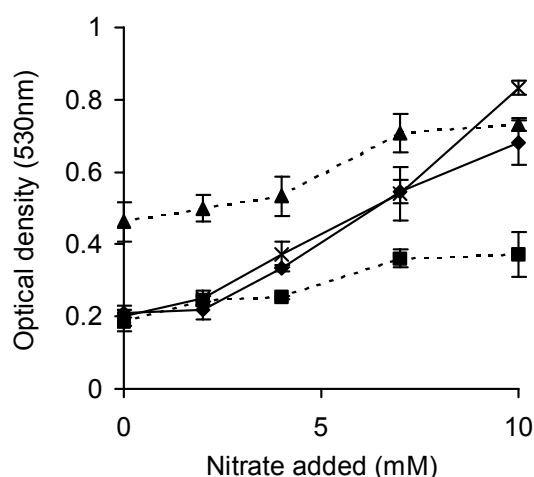


FIG. 3. Patterns of nitrate-dependent growth of *Bacillus* sp. C2 (x), *Bacillus* sp. C24 (▲), *Bacillus* sp. C8 (■) and *Bacillus* sp. B9 (x). Solid lines represent growth patterns of vigorous denitrifiers. Broken lines represent growth patterns of DNRA bacteria.

From Griffith soil, of the 220 isolates that were able to grow anaerobically on the media used (NB and NB plus glucose), 129 were obligate anaerobes, forty-one were nitrite accumulators, forty-seven were DNRA bacteria and six were denitrifiers (Table III). From Yangzhou soil, 113 isolates were able to grow anaerobically, among which fifteen were obligatory anaerobes, forty-nine nitrite accumulators, six DNRA bacteria and thirty-four denitrifiers. DNRA bacteria accounted for 21% of total isolates in Griffith soil and 5.3% in Yangzhou soil, whereas denitrifiers accounted for 2.7% and 30% of total isolates in the two soils, respectively. Most of the DNRA and denitrifying isolates were spore-formers (Table III).

Growth of all of the denitrifying isolates increased linearly with increasing  $\text{NO}_3^-$  addition to NB medium, while growth of most of the DNRA isolates did not. A few examples are shown in Fig. 3. The growth patterns met the criteria proposed by Mahne and Tiedje [16]. The pattern of  $\text{NO}_3^-$  reduction during cell growth of a DNRA isolate (*Bacillus* sp. C24) in  $\text{NH}_4^+$ -free medium is shown in Fig. 4. This pattern was similar to those reported for other DNRA bacteria [11,17–19].

#### 4. DISCUSSION

Two soils significantly differed in their potential for the DNRA process. Griffith soil converted 14.5% of  $\text{NO}_3^-$  to  $\text{NH}_4^+$ -N plus organic-N without an exogenous C source (Table I), which is, to our knowledge, the highest value reported in an agricultural soils under similar experimental conditions.

TABLE III. NUMBER OF ISOLATES CHARACTERIZED FOR THEIR NITRATE REDUCTION AND ANAEROBIOSIS

Soil	Type of isolate			
	Nitrate accumulator	DNRA bacterium	Denitrifier	Obligatory anaerobe
Griffith soil	41 (38) <sup>a</sup>	47 (46)	6 (4)	129
Yangzhou soil	49 (43)	6 (6)	34 (26)	15

<sup>a</sup>Number of spore-forming isolates in parentheses.

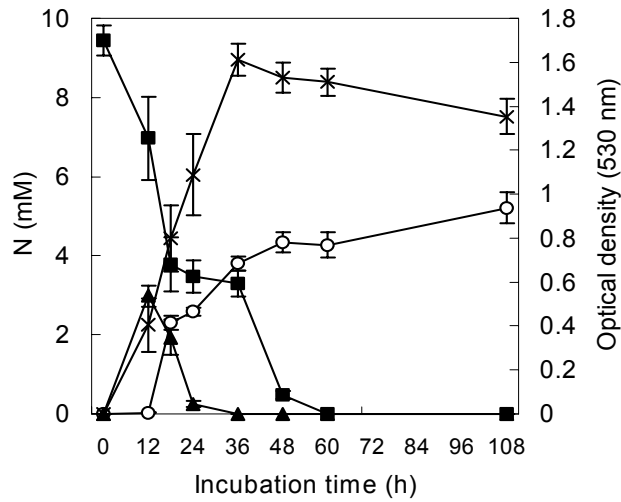


FIG. 4. Growth and nitrate reduction by *Bacillus* sp. C24 in ammonium-free medium ( $\circ$  :  $\text{NH}_4^+$ ;  $\blacktriangle$  : nitrite;  $\blacksquare$  : nitrate;  $\times$ : optical density).

Data reported by others are listed in Table IV; it is noteworthy that the data of Stanford et al. [20] were higher than those obtained in the present study, but those experimental conditions involved pre-incubation with glucose. The high recovery, 21%, reported by Chen et al. [6] for the same soil was due to pre-incubation (3 days) and the very low  $\text{NO}_3^-$  concentration used in that study ( $2.5 \text{ mg N kg}^{-1}$ ), probably giving a high C:N ratio that would be expected to favour DNRA [2,21,22]. The remarkably high DNRA activities in the Griffith soil may have agronomic importance, warranting further study.

TABLE IV. REPORTED RECOVERIES OF  $^{15}\text{N}$ -LABELLED NITRATE AS  $\text{NH}_4^+\text{-N}$  AND ORGANIC-N IN ANAEROBICALLY INCUBATED SOILS WITHOUT EXOGENOUS CARBON

Brief description of experimental conditions	Recovered as $\text{NH}_4^+\text{-N}$ (%)	Recovered as organic-N (%)	Highest recovery (%)	Sources
Three soils including one used in this study, fresh, pH 6.8–8.4, $2.5 \text{ mg N kg}^{-1}$ nitrate added, incubated at $30^\circ\text{C}$ for 6–54 h	5.5–14.5	5.0–7.8	21.1	[6]
Two air-dried soils, pH 5.5–8.0, $300 \text{ mg N kg}^{-1}$ nitrate added, incubated at $30^\circ\text{C}$ for 6 d	1.2–1.3	1.0–5.6	6.8	[7]
Three soils, heat-shocked or not, fresh and air-dried, pH 6.8, $80 \text{ mg N kg}^{-1}$ nitrate added, incubated at $28^\circ\text{C}$ for 5 d			1.1–4.8	[4]
One soil, fresh, pH 7.4, $200 \text{ mg N kg}^{-1}$ nitrate added, incubated at $25^\circ\text{C}$ for 3 d	0.13	6.8	6.9	[23]
One soil, air-dried and 1 d preincubated anaerobically without glucose, $100 \text{ mg N kg}^{-1}$ nitrate added, incubated at $30^\circ\text{C}$ for 4 d	0.9–1.8	Below detection limit of 1.3	3.1	[1]
Two soil, pre-incubated with glucose, pH 6.2–6.5, $185 \text{ mg N kg}^{-1}$ nitrate added, incubated at $35^\circ\text{C}$ for 1 d, tube stoppered under normal atmosphere	8.5–19.4	17.2–18.2	37.6	[20]

Reducing agents increased the partitioning of  $\text{NO}_3^-$  to DNRA (Fig. 1A) in Griffith soil, which is generally to be expected and is consistent with results reported by others [1]. This effect, however, was not observed in Yangzhou soil. The reason is not clear. Fazzolari et al. [22] reported that DNRA activity was, in fact, less sensitive than denitrification to inhibitory effects of  $\text{O}_2$ . The effects of reducing agents on DNRA may, therefore, vary with soil and bacterial species.

Glucose significantly affected DNRA (Fig. 1B), as previously reported [1,4,22,23]. The main effect was increase in recovery of applied  $^{15}\text{N}$ -nitrate in the organic-N pool. For Griffith soil with *L*-cysteine,  $^{15}\text{N}$  recovery in the  $\text{NH}_4^+$  pool was lower with glucose treatment than in non-glucose-treated samples (Fig. 1A), indicating that  $\text{NH}_4^+$  assimilation occurred. Nitrate assimilation was unlikely to occur because 100 mg N  $\text{kg}^{-1}$  of non-labelled  $\text{NH}_4^+$  were added, both in pure culture of bacteria [24] and in soils [8]. Therefore, the  $^{15}\text{N}$  recovered in the organic-N pool must have come from the DNRA pathway. Mole ratio of glucose-C: $\text{NO}_3\text{-N}$  in the present study was 12:1. At this C:N ratio, denitrification was the dominant process in Yangzhou soil, but was not in the Griffith soil. In all non-glucose treatments,  $\text{N}_2\text{O}$  oxide accumulated within 1.5 days incubation, and decreased thereafter. The opposite was true for glucose treatment. The consumption of  $\text{N}_2\text{O}$  is explained only in terms of denitrification.

Straw mulching is a common practice in the Griffith locality. In Yangzhou, however, soils receive little organic materials except crop residues, mainly roots. The soil at Yangzhou is cropped all year. Continuous straw mulching not only provides more available carbon for DNRA, but also supports large populations of heterotrophs, including denitrifiers and DNRA bacteria. Straw management might partially explain the higher DNRA activity in Griffith soil.

The microbial study indicated that abundance (Fig. 2) and type (Table IV) of  $\text{NO}_3^-$  reducers in the two soils were very different, although it was difficult to assign specific ecological roles. Most DNRA and denitrifying bacteria isolated and characterized (a few examples are shown in Figs. 3 and 4) were spore-formers, consistent with previous reports [4,25,26].

## ACKNOWLEDGEMENTS

The authors express appreciation to Dr. Elizabeth Humphreys (CSIRO Land and Water–Griffith Laboratory) for her assistance in sampling Griffith soil; Ron Teo and Raffael Timpano for their technical assistance; Dr. Peter Janssen for comments and providing culture medium; Professor Z.M. Yan (Yangzhou Environment Monitoring Station, Jiangsu, China) for his friendly help; and the Open Laboratory of Crop Physiology, Yangzhou University, for the GC and other facilities.

## REFERENCES

- [1] BURESH, R.J., PATRICK, W.H., Nitrate reduction to ammonium in anaerobic soils. *Soil Sci. Soc. Am. J.* **42** (1978) 913–918.
- [2] TIEDJE, J.M., et al., Denitrification: ecological niches, competition and survival, *Antonie van Leeuwenhoek J. Microbiol.* **48** (1982) 569–583.
- [3] TIEDJE, J.M., “Ecology of denitrification and dissimilatory nitrate reduction to ammonium”, *Biology of Anaerobic Microorganisms* (ZEHNDER, A.J.B., Ed.), John Wiley & Sons, Inc. New York (1988) 179–244.
- [4] CASKEY, W.H., TIEDJE, J.M., Evidence for *Clostridia* as agents of dissimilatory reduction of nitrate to ammonium in soils, *Soil Sci. Soc. Am. J.* **43** (1979) 931–936.
- [5] YIN, S.X., et al., Reduction of nitrate to ammonium in selected paddy soils of China, *Pedosphere* **8** (1998) 221–228.
- [6] CHEN, D.L., et al., Estimation of nitrification rates in flooded soils, *Microbial Ecol.* **30** (1995) 269–284.
- [7] CHEN, D.L., et al., Distribution of reduced products of  $^{15}\text{N}$ -labelled nitrate in anaerobic soils, *Soil Biol. Biochem.* **27** (1995) 1539–1545.
- [8] RICE, C.W., TIEDJE, J.M., Regulations of nitrate assimilation by ammonium in soils and in isolated soil microorganisms, *Soil Biol. Biochem.* **21** (1989) 579–602.

- [9] KASPAR, H.F., TIEDJE, J.M., "Anaerobic bacteria and processes", Methods of Soil Analysis, Part 2, Microbiological and Biochemical Properties (WEAVER, R.W., et al., Ed.), Soil Science Society of America, Madison (1994) 223–243.
- [10] TIEDJE, J.M., "Denitrifiers" Methods of Soil Analysis, Part 2, Microbiological and Biochemical Properties (WEAVER, R.W., et al., Ed.), Soil Science Society of America, Madison (1994) 245–267.
- [11] FAZZOLARI, E., et al., Dissimilatory ammonium production vs denitrification in vitro and in inoculated agricultural soil samples, Can. J. Microbiol. **36** (1990) 786–793.
- [12] CHEN, D.L., et al., Nitrogen transformation in flooded soil in the presence and absence of rice plant: 2. Denitrification, Nutr. Cycl. Agroecosyst. **51** (1998) 269–279.
- [13] MULVANEY, R.L., BOAST, C.W., Equations for determination of nitrogen-15 labelled dinitrogen and nitrous oxide by mass spectrometry, Soil Sci. Soc. Am. J. **50** (1986) 360–363.
- [14] DANIELS, L., et al., "Chemical analysis", Methods for General and Molecular Bacteriology (GERHARDT, P., et al., Eds.), American Society for Microbiology, Washington, DC (1994) 512–554.
- [15] THAUER, R.K., et al., Energy conservation in chemotrophic anaerobic bacteria, Microbiol. Rev. **41** (1977) 100–180.
- [16] MAHNE, I., TIEDJE, J.M., Criteria and methodology for identifying respiratory denitrifiers, Appl. Environ. Microbiol. **61** (1995) 1110–1115.
- [17] FAZZOLARI, E., et al., Nitrate reduction to ammonia: a dissimilatory process in *Enterobacter amnigenus*, Can. J. Microbiol. **36** (1990) 779–785.
- [18] SMITH, M.S. Dissimilatory reduction of  $\text{NO}_2^-$  to  $\text{NH}_4^+$  and  $\text{N}_2\text{O}$  by a soil *Citrobacter* sp., Appl. Environ. Microbiol. **43** (1982) 854–860.
- [19] SMITH, M.S., ZIMMERMAN, K., Nitrous oxide production by nondenitrifying soil nitrate reducers, Soil Sci. Soc. Am. J. **45** (1981) 865–871.
- [20] STANFORD, G.L., et al., Denitrification and associated nitrogen transformation in soils, Soil Sci. **120** (1975) 147–152.
- [21] Cole, J.A., Brown, C.M., Nitrate reduction to ammonia by fermentative bacteria: A short circuit in the biological nitrogen cycle, FEMS Microbiol. Lett. **7** (1980) 65–72.
- [22] FAZZOLARI, et al., Simultaneous effects of increasing levels of glucose and oxygen partial pressures on denitrification and dissimilatory nitrate reduction to ammonium in repacked soil cores, Eur. J. Soil Biol. **34** (1998) 47–52.
- [23] deCATANZARO, J.B., et al., Denitrification vs dissimilatory nitrate reduction in soil with alfalfa, straw, glucose and sulfide treatments, Soil Biol. Biochem. **19** (1987) 583–587.
- [24] SIAS, S.R., INGRAHAM, J.L., Isolation and analysis of mutants of *Pseudomonas aeruginosa* unable to assimilate nitrate, Arch. Microbiol. **122** (1979) 263–270.
- [25] LI, Z.G., et al., Numbers, compositions and enzyme activities of denitrifiers in paddy soils of Taihu Lake district, Acta Pedologica Sinica **26** (1989) 79–86.
- [26] GARCIA, J.-L. Analyse de différents groupes composant la microflore dénitrifiante de sols de rizière du Sénégal, Ann. Microbiol. (Inst. Pasteur) **128A** (1977) 433–446.



## GREEN-MANURE (*Gliricidia sepium*) EFFECTS ON GROWTH AND YIELD OF COFFEE AND QUANTIFICATION OF NITROGEN RECOVERY USING $^{15}\text{N}$ DILUTION

W.D.L. GUNARATNE, A.P. HEENKENDA

Department of Export Agriculture,  
Research Station, Matale, Sri Lanka

### Abstract

A field study was conducted on a Typic Rhodustalfs to assess the effects of various rates of *Gliricidia sepium* prunings on growth and yield of coffee and on soil chemical properties. Nitrogen transfer from the green manure (GM) to coffee was estimated using the  $^{15}\text{N}$ -dilution technique. All growth parameters and yield of coffee were significantly increased by green manure application over the control, but no significant differences were found among 10, 15 and 20 kg/plant/yr of GM. A nearly five-fold yield increase was observed even at the fifth crop, with GM. Percentage of N derived from GM in the coffee ranged from 50 to 64, with values equivalent to 11.6, 13.5 and 11.2 g N/plant for the 10-, 15- and 20-kg treatments, respectively. Increases in total N, exchangeable K, Mg and Zn, soil pH, and organic matter content were obtained even at lower soil profiles. Total-N content in 0 to 10 cm increased from 0.063 to 0.120, 0.227 and 0.301% with 10-, 15- and 20-kg GM treatments. Similarly, four- to seven-fold increases in available K, a half- to two-fold increases in available Mg, and two- to two-and-a-half-fold increases were observed in organic C.

### 1. INTRODUCTION

Although agroforestry is not new to farming systems in most countries of Asia, the possible benefits from them are poorly utilized due to inadequate awareness. Beneficial effects from such systems include maintenance of soil fertility, arresting soil erosion, insurance against risk, and other economic benefits accruing from the production of fuel wood, fodder, timber, medicines, and fruits. Low productivity associated with degradation of soil physical, chemical and biological properties resulting from unprotected cultivated soil is one of the critical problem in central region of Sri Lanka, where land topography varies from rolling to hilly. Cultivation of commercial crops, such as tea, coffee, cocoa and black pepper, is common. Unpredictable rainfall pattern and high cost of fertilizer are two major contributing factors for the low productivity of most of the small- to medium-scale farms. With respect to N inputs, use of biological  $\text{N}_2$  fixation associated with leguminous plants has a great potential for agriculture [1–3]. The global contribution of N from biological  $\text{N}_2$  fixation has been estimated to range from 129 to  $170 \times 10^6$  t N/yr [4,5], which is two- to three-fold higher than inputs from fertilizers ( $65 \times 10^6$ ) [5].

Proper tree management and efficient utilization of tree products, within the system, are essential for sustainability. Increases in yields accrue when beneficial effects are greater than competitive effects for a single factor or combination of factors. Periodical pruning of hedgerow trees—essential to prevent shading and to reduce competition with the companion crop—produces large quantities of mulch and green manure. In addition to recycling nutrients, these suppress weeds, conserve soil moisture, control soil erosion, reduce soil temperatures, and increase soil aggregation, thereby improving soil structure [6,7]. Release of nutrients, especially N, from organic residues depends on the chemical and physical properties, environmental conditions and the decomposer communities [8]. To a great extent, rate and extent of decomposition are determined by the material's initial content of N, lignin, and polyphenol oxidase [9]. On the other hand, poor efficiency of N use from the residues is associated with gaseous and leaching losses due to lack of synchrony between crop-N demand and N release. Alternatively, some of the released N may be incorporated into the soil organic matter. Especially under perennial cropping systems, important factors are: total biomass production, the period during which the maximum quantity is recycled within the system, the period of maximum demand for nutrient by the companion crop, and the nutrient-release pattern.

Coffee is an important beverage crop grown in the central region of Sri Lanka over an area of 15,500 ha. It is cultivated either as an intercrop with another commercial perennial crop such as coconut or as a monocrop with shade-tree species, e.g. *Gliricidia sepium*, *Erythrina* spp. and *Gravelia robusta*. National annual average productivity (156 kg/ha) is far below the potential of 1,000 kg/ha [1]. Fertilizer is the main material input, but most growers do not apply adequate amounts due to high cost. This situation compels us to find alternatives for inputs while conserving resources [1,2,7]. *Gliricidia sepium* (Jacquin) Kunth ex Walp. (gliricidia) is commonly grown with coffee as a shade tree; its leaves and tender stems are rich in N and other essential plant nutrients [3,10].

This paper describes the effects of field application of various rates of green manure (GM) from gliricidia on growth and yield of coffee, N contributions from the GM to the coffee plants, and effects on soil chemistry.

## 2. MATERIAL AND METHODS

The experiment was conducted at the Research Station of the Department of Export Agriculture, located in Matale, Sri Lanka (350 m AMSL, 72°8'N and 80°38'E) on Typic Rhodudalfs (texture clay loam to loam, bulk density 0.9–1.1 g/cm<sup>3</sup>, organic C content 1–2%, pH 4.3–5.5, CEC 10–15 cmol/kg, total N content 0.12–0.18%, exchangeable K 0.012–0.018% and exchangeable Mg 0.025–0.036%). Four-month-old seedlings of coffee cv. Catimor were field planted at 1.2×1.2-m spacing in 45×45×45-cm pits filled with topsoil and 10 kg of farmyard manure. Four months before planting of coffee, gliricidia was established at 2.4×2.4-m spacing using 2-m-long stem cuttings to ensure adequate shade for the coffee. The experiment consisted with four treatments: 0, 10, 15, and 20 kg/plant/yr of fresh gliricidia prunings (in four split applications). Treatments were replicated four times in a RCBD design. Each plot consisted with twelve coffee plants and two central plants of the plots were used as <sup>15</sup>N isotope microplots to quantify the rate of N transfer to coffee through applied green manure, using the isotope-dilution technique. Isotope microplots were separated from the balance of the plants using two layers of black polythene inserted to a depth of 0.75 m. From the third month after field planting, 10.39 atom % <sup>15</sup>N ammonium sulphate was applied at the rate of 20 kg/ha in four split applications during the first year of growth. Gliricidia prunings, collected from each of the plots, were applied to the same plot, preferentially to the isotope microplots, with the balance of the green manure requirement, if any, collected from trees grown along the boundaries. As indicated, green manure was applied in four equal splits as a mulch at the base of coffee plants. Plots were kept free of weeds throughout the experiment period. All the weeds from isotope microplots were returned to the same plots to prevent isotope contamination.

Growth measurements of the coffee plants were made at 9 and 15 months after field planting. At the end of the second year, when plants were ready for the first harvest, coffee plants from the isotope microplots were cut at 3 cm from the ground level. Leaves, trunk, twigs and berries were separated to record fresh weight. Representative subsamples were dried in an oven at 70°C to a constant weight. Subsamples from them were used to determine dry weights of the plant parts and to analyse for N content and <sup>15</sup>N atom excess. Percentage of N derived from green manure was calculated based the following isotope-dilution equation:

$$\%N \text{ from GM} = \left[ 1 - \frac{(^{15}\text{N a.e. in GM-treated plants})}{(^{15}\text{N a.e. in control plants})} \right] \times 100$$

where

GM is green manure.

Soil samples were taken at three depths (0–10, 10–20, and 20–30 cm) and determinations made of changes in pH (1:2.5 soil:H<sub>2</sub>O), total N, exchangeable P (Olsen), NH<sub>4</sub>OAC extractable K, Mg, Cu, and Zn, as well as organic matter content after 36 months of field establishment.

Applications of GM were continued as described above to observe long-term effects on coffee, and improve the reliability of recommendations for growers. Yield data were collected annually (5 yr) and recorded as dry parchment coffee.

### 3. RESULTS AND DISCUSSION

Positive effects of GM on the growth of the coffee plants were clear within 9 months (Table I). Except for number of lateral branches, growth parameters were significantly increased over those for the controls. From the 15<sup>th</sup> month onwards, number of laterals also was significantly increased. No significant differences were observed among the three rates of application of GM.

Plant dry weights recorded at the end of the second year indicated significant effects of the GM treatments, except for trunk and laterals (Table II). Even at this stage, no significant differences were observed among GM treatments. No significant differences were observed for %N for any of the plant parts. However, total-N yields of the leaves were significantly increased by GM application. This was due to high leaf and berry yields, reflecting increased vigour of the GM-treated plants.

Values for <sup>15</sup>N a.e. in all the parts of the GM-treated plants were significantly lower than those of the controls, confirming that, in addition to soil N, these plants had assimilated N from the GM (Table III). The percentage of N derived from GM (PND) showed significant differences for leaves and berries. In the case of leaves, the 20-kg rate produced lower values for PND than did 10 kg, which is hard to explain. This trend prevailed throughout the experiment; the 20-kg-treated plants were consistently less vigorous, though the differences were not significantly different. However, in the case of berries, the 20-kg treatment scored highest for PND. On average, 48 to 64% of N was provided by the green manure.

Of total plant N, 57, 59 and 55% originated from the 10-, 15- and 20-kg GM treatments, respectively, i.e. 11.6, 13.5 and 11.2 g N/plant, respectively (Table III). Cadisch et al. [9] reported recoveries of 53 to 69% of N by maize from gliricidia and *Leucaena* prunings. This indicates that nutrient release from crop residues and green manure is a long-term process; continuous application of green manure for perennial crops like coffee is likely to sustain the productivity of the soil.

TABLE I. EFFECTS OF GLIRICIDIA GREEN MANURE ON GROWTH OF COFFEE

GM treatment (kg/plant/yr)	Plant height		Lateral spread		Laterals/plant		Leaves/plant	
	9 map <sup>a</sup>	15 map	9 map	15 map	9 map	15 map	9 map	15 map
	———— (cm) ———		———— (cm) ———					
0	50.9	75.4	40.4	66.8	10.7	20.3	106	242
10	54.8	94.4	50.3	95.5	12.4	26.8	159	483
15	56.5	92.3	52.0	92.8	12.7	26.2	156	459
20	52.3	90.0	46.0	90.3	11.6	25.2	131	448
	***	***	***	***	NS	**	**	***
LSD <sub>0.05</sub>	5.59	11.65	6.97	14.2	8.21	2.88	9.34	101.9
CV%	4.79	8.27	5.26	10.3	1.55	7.32	20.6	15.6

<sup>a</sup>Months after planting.

TABLE II. EFFECTS OF GLIRICIDIA GREEN MANURE ON DRY WEIGHT, %N CONCENTRATION, AND TOTAL-N YIELD OF COMPONENTS OF COFFEE PLANTS

GM treatment (kg/plant/yr)	Leaves			Trunk			Laterals			Berries			Total	
	Wt. (g)	N (%)	N (g)	Wt. (g)	N (%)	N (g)	Wt. (g)	N (%)	N (g)	Wt. (g)	N (%)	N (g)	Wt. — (g)	N —
0	181	2.29	4.00	86.2	0.49	0.55	82.9	0.99	0.79	279	1.35	3.86	630	9.20
10	429	2.49	9.59	157	0.66	0.05	193	1.09	2.24	611	1.36	8.32	1,390	21.2
15	472	2.48	10.4	186	0.71	0.94	205	1.22	1.78	634	1.38	8.47	1,497	21.6
20	426	2.42	9.58	151	0.56	0.89	177	1.20	2.13	540	1.39	7.49	1,294	20.1
LSD <sub>0.05</sub>	**	NS	***	NS	NS	NS	NS	NS	NS	***	NS	**	ND <sup>a</sup>	ND
CV%	20.4	13.7	17.6	43.5	27.5	51.6	41.3	18.1	49.9	19.8	23.1	29.8		

<sup>a</sup>Not determined.

TABLE III. <sup>15</sup>N a.e., %N DERIVED FROM GREEN MANURE (PND) AND TOTAL AMOUNT OF N DERIVED FROM GREEN MANURE (TN) IN COMPONENTS OF COFFEE PLANTS

GM treatment (kg/plant/yr)	Leaves			Trunk			Laterals			Berries		
	<sup>15</sup> N a.e. (%)	PND (%)	TN (g/plant)	<sup>15</sup> N a.e. (%)	PND (%)	TN (g/plant)	<sup>15</sup> N a.e. (%)	PND (%)	TN (g/plant)	<sup>15</sup> N a.e. (%)	PND (%)	TN (g/plant)
0	0.5807	—	—	0.5703	—	—	0.5257	—	—	0.6095	—	—
10	0.2529	62.1	5.93	0.2468	60.3	0.466	0.2287	56.4	1.28	0.3081	48.3	3.88
15	0.2529	63.7	6.61	0.2569	55.2	0.519	0.2449	53.4	0.936	0.2332	63.0	5.43
20	0.2688	53.5	4.92	0.2734	53.5	0.474	0.2613	50.8	1.083	0.2198	63.8	4.76
LSD <sub>0.05</sub>	***	**	***	***	NS	NS	***	NS	NS	***	***	NS
CV%	0.1024	8.02	0.893	0.0949	10.2	0.552	0.0695	12.4	1.079	0.0596	7.58	2.37
	18.9	7.75	8.86	17.6	10.4	45.5	13.8	13.4	56.8	11.0	7.51	29.2

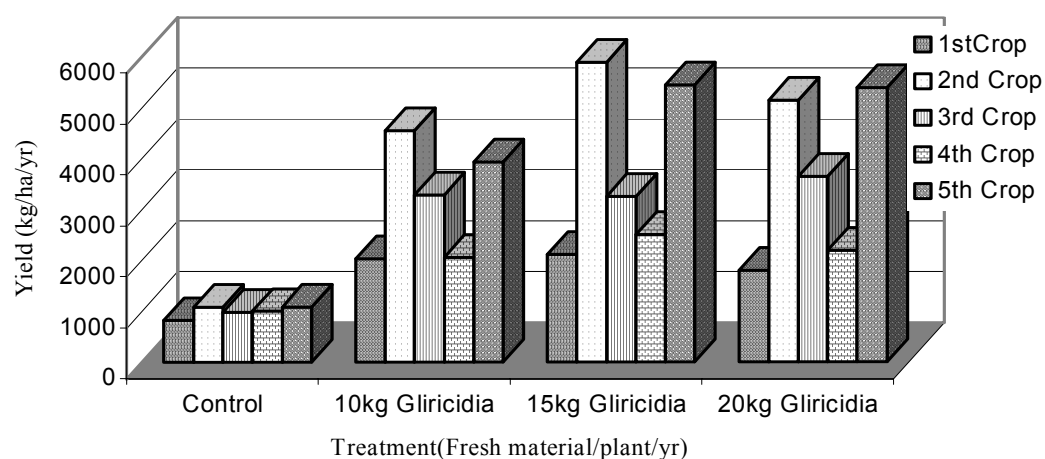


FIG. 1. Effects of rate of application of GM (*gliricidia*) prunings on coffee yield.

Coffee-berry yields are shown in Fig. 1. All five harvests indicated the same trend, and the controls gave significantly lower yields than did the GM treatments. The differences in berry yields among GM rates were not significant ( $P=0.05$ ) throughout the recording period. Yields were high for the second crop, declined for the third and fourth, then improved for the fifth in 1999. A trend of decreasing yields is common for var. Catimor. According to Sumanasena and Wickramasinghe [11], a third crop of this variety of coffee yielded only 257g/plant (856kg/ha). Applications of green manure may maintain canopy vigor, arresting or decreasing defoliation during the dry months through soil-moisture conservation.

Green manure improved all the soil chemical properties tested, in comparison to the control values (Table IV). Increases in total N, exchangeable K, Mg and Zn, soil pH and organic matter content were observed even at lower soil profiles. Total N contents at 0 to 10 cm increased from 0.063 to 0.120, 0.227, and 0.301%, with 10-, 15-, and 20-kg treatments, respectively. Similarly, four- to seven-fold increases in available K, 0.5- to two-fold increases in available Mg, and two to 2.5-fold increases in organic C were observed. The results indicate that the application of *gliricidia* as a green manure is a viable and economical, though it requires more labour than mineral fertilizer application. However, when savings in fertilizer costs are considered in conjunction with beneficial effects, this practice holds the promise of being sustainable.

TABLE IV. EFFECTS OF GLIRICIDIA GREEN MANURE ON SOIL-CHEMICAL PROPERTIES

GM (kg/pl/yr)	Depth (cm)	pH	N (%)	O.C. (%)	P (ppm)	K (%)	Mg (%)	Cu (ppm)	Zn (ppm)
0	0–10	4.04	0.063	1.40	20	0.004	0.023	19.5	2.4
	10–20	4.00	0.031	2.00	30	0.002	0.021	19.1	1.75
	20–30	4.00	0.024	1.33	20	0.004	0.021	18.0	1.83
10	0–10	4.37	0.120	2.61	20	0.016	0.034	19.0	3.1
	10–20	4.18	0.140	2.52	10	0.007	0.028	19.3	2.4
	20–30	4.16	0.180	1.50	10	0.004	0.039	19.2	2.1
15	0–10	4.36	0.227	3.53	30	0.024	0.033	16.6	2.5
	10–20	4.15	0.072	2.20	20	0.014	0.036	17.8	3.1
	20–30	4.19	0.034	1.47	20	0.007	0.035	16.6	3.8
20	0–10	4.77	0.301	3.54	20	0.030	0.046	20.0	3.5
	10–20	4.45	0.179	2.20	20	0.020	0.036	20.8	2.1
	20–30	4.27	0.027	2.07	20	0.014	0.036	19.1	2.1

#### 4. CONCLUSIONS

The results suggest that gliricidia prunings can be effective as a source of nutrient for coffee. An application of 10 kg of fresh material per plant per year appeared to be adequate under the prevailing conditions. Considering that most soils in the coffee-growing area of Sri Lanka are less productive than the soil used in this experiment, a recommendation of 10 kg/plant/yr would be generally applicable, and would supply about 50% of nutrient requirements. Further studies on soil-moisture retention and structure improvement are required to fully understand the beneficial effects of gliricidia prunings as a green manure.

#### ACKNOWLEDGEMENTS

The authors are grateful to the International Atomic Energy Agency for technical support for this research programme. Comments and suggestions made by Dr. Saliya Kumarasinghe, Dr. N Sanginga and Dr. Gamini Keerthisinghe are greatly appreciated. Also, we extend our thanks to Dr. C.S. Smith of CSIRO, Canberra, for facilities provided to analyse samples for  $^{15}\text{N}$  a.e.

#### REFERENCES

- [1] PEOPLES, M.B., CRASWELL, E., Biological nitrogen fixation: Investments, expectations and actual contribution to agriculture, *Plant Soil* **141** (1992) 13–39.
- [2] LIYANAGE, M., et al., Biological nitrogen fixation in four *Gliricidia sepium* genotypes, *Plant Soil* **161** (1994) 267–274.
- [3] McDONAGH, J.F., et al., Grain legumes and green manures as pre-rice crops in northeast Thailand, *Plant Soil* **177** (1995) 111–126.
- [4] Burns, R.C., Hardy, R.W.F., *Nitrogen Fixation in Bacteria and Higher Plants*, Springer-Verlag, Berlin (1975).
- [5] PAUL, E.A., “Towards the year 2000: Directions for future nitrogen research”, *Advances in Nitrogen Cycling in Agricultural Ecosystems* (Wilson, J.R., Ed.), CAB International, Wallingford (1988) 417–425.
- [6] AMARA, D.S., et al., Nitrogen contribution by multipurpose trees to rice and cowpea in an alley cropping system in Sierra Leone, *Agroforestry Syst.* **34** (1996) 119–128.
- [7] CAMPBELL, C.A., et al., Managing nitrogen for sustainable crop production, *Fertilizer Res.* **42** (1995) 277–296.
- [8] HEAL, O.W., et al., “Plant litter quality and decomposition: An historical overview”, *Driven by Nature* (CADISCH, G., GILLER, K.E., Eds.), CAB International, Wallingford (1997) 3–32.
- [9] CADISCH, G., et al., N recovery from legume prunings and priming effects are governed by the residue quality, *Plant Soil* **205** (1998) 125–134.
- [10] GUNARATNE, W.D.L., HEENKENDA, A.P., “Effect of different pruning intervals on biomass production of *Gliricidia sepium* and yield of *Piper nigrum* L.”, *Proc. of the 4<sup>th</sup> Regional Workshop on MPTs* (1993) 102–109.
- [11] SUMANASENA, H.A., WICKRAMASINGHE, P.J., “Effect of flower bud removal on long term yield performance of catimor coffee”, *Annual Report, Department of Export Agriculture P2, Matale, Sri Lanka, Department of Export Agriculture, Matale* (1996).

# NITROGEN RECOVERY BY MAIZE FROM A COMBINATION OF MUCUNA RESIDUES AND INORGANIC N IN THE DERIVED SAVANNAH OF SOUTHERN BENIN USING $^{15}\text{N}$

P. HOUNGNANDAN

Institut National des Recherches Agricoles du Benin (INRAB),  
Cotonou, Benin

N. SANGINGA, B. VANLAUWE

International Institute of Tropical Agriculture (IITA),  
Ibadan, Nigeria

O. VAN CLEEMPOT

Faculty of Agricultural and Applied Biological Sciences,  
University of Ghent, Ghent, Belgium

## Abstract

Quantification of uptake of N from *Mucuna pruriens* residues by maize in combination with inorganic N (ammonium sulphate) is essential for the development of management practices that optimize N-use efficiency in low-input tropical cropping systems. The recovery of N from  $^{15}\text{N}$ -labelled mucuna residues was evaluated in a microplot experiment on a degraded soil in the derived savannah at Niaouli, southern Benin Republic. Results showed that when 90 kg ha<sup>-1</sup> of labelled N as mucuna residues were applied, or 45 kg N ha<sup>-1</sup> as residues labelled or unlabelled were combined with the same rate of ammonium sulphate, the total maize DM and N yields were found to be significantly higher ( $P < 0.05$ ) than when fertilizer-N was applied alone. The yields of the treatments with mucuna residues averaged 4,504 kg ha<sup>-1</sup> DM with accumulation of 57 kg N ha<sup>-1</sup>. These results show that although the labelled mucuna residues applied at 90 kg N ha<sup>-1</sup> gave the highest total maize yield, the proportion of labelled N from the residues was very low according to the  $^{15}\text{N}$  data. However, higher values for percent recovery of input were found using the difference method.

## 1. INTRODUCTION

The combined use of mineral and organic sources of nutrients has been a major emphasis of research within the Soil Fertility Network [1] and has been proposed as an attractive management option to solve problems of N deficiency in degraded soils [2]. Previous studies have revealed that, in the first crop, the recovery of N from organic residues is very variable, and less than 20% [3–7]. Several factors have been identified as important influences on rate of decomposition of organic residues and the transfer of N from legumes to other crops [8].

In a study of potential benefits from interactions between mineral and organic nutrient sources, Giller et al. [9] observed that the most important mechanisms for interactions are considered to be:

- effects of the addition of available C on the mineralization / immobilization of N,
- effects of the addition of available C on the availability of P, and
- effects of P on the capture of mineral N in soil due to increased crop-root growth or due to increased  $\text{N}_2$  fixation in legumes.

These authors observed also other potential interactions, but evidence for their occurrence is sparse, tending to be limited to isolated or extreme cases, and their short-term effects on soil fertility are likely to be small [10].

Interactions have a number of possible mechanisms that can be broadly grouped into direct effects on nutrient dynamics and those that affect the plant's ability to capture nutrients. Amongst them are: regulators of mineralization-immobilization of N, C:N ratio, microbial activity, lignin and polyphenol contents of the plant residues, water availability, and temperature [9,11,12]. Palm et al. [10]

summarized the concept through numerous trials by comparing the yields from a given amount of inorganic fertilizer (A), an organic material (B), and their interactions (A + B), and showed that, in many situations, (A + B) produced higher yields than did A or B alone. The authors concluded that it should not be surprising that the combination does better because more total nutrients are added.

These results have been confirmed by research in the south of Benin. Iwuafor et al. [13] observed a maize-yield increase of 91%, by combining 45 kg N ha<sup>-1</sup> as cowpea residue with 45 kg N ha<sup>-1</sup> as urea in a multi-locational trial on twenty-four farmers' fields in the derived savannah (DS). With residues from *Leucaena leucocephala* and *Senna siamea* in Sekou (southern Benin), the combined application of organic N and urea-N was shown to give significantly higher maize yields than the application of only organic matter applied at the same N rate [7]. Work by Houngnandan et al. [14] in the DS confirmed these findings and indicated that mucuna residues together with urea-N had positive effects on maize yields and N-use efficiency. These effects were highly significant when mucuna residues were incorporated into the soil and less so when they were applied to the surface; however, no study was done to determine the proportion of applied N from mineral fertilizer or mucuna residue that was recovered by the maize. Therefore, a microplot experiment was carried out on a degraded soil in the DS at Niaouli in southern Benin to quantify the contribution of N from *Mucuna pruriens* residues to maize.

## 2. MATERIALS AND METHODS

### 2.1. Site description

The trial was carried out at the Niaouli Experimental Station in 1998 (06°40'N, 02°10'E, altitude 100 m) in the DS benchmark area of southern Benin on a site referred to as "terre de barre" soils, a common local name for the various red soils developed on the "continental terminal" formation. The "terre de barre" is characterized by an increasing clay content (kaolinite) with depth, and is formed on oligocenian sea-terrace (parental material is sedimental origine-continental terminal). The "terre de barre" is moderately slippery on the surface after rainfall and highly weathered, as much as 10 m deep, well drained, sesquioxide rich, and mainly kaolinitic. The soil of the site is sandy over sandy clay loam, classified as a Rhodic Ferralsol [15]. Between 0- and 15-cm depth, it had an organic C content of 0.37%, a total N content of 0.04% and a pH (KCl) of 4.4.

The experiment was established on a degraded soil, some properties of which are given in Table I. Although the zone is characterized by a sub-equatorial humid hot climate with a bimodal rainfall distribution, the rainfall distribution was especially weak in 1998. The field trial was irrigated for most of the growing period.

### 2.2. Experimental

The <sup>15</sup>N-labelled mucuna residues were produced under field conditions at Niaouli, 15 June to 10 October, 1997. Mucuna was planted in two bands of ten plots of 2 m<sup>2</sup> each. One band was labelled two weeks after planting using (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> at 90 kg N ha<sup>-1</sup> with an enrichment of 10.16 atom % <sup>15</sup>N, while a second band received unlabelled (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> at the same rate.

TABLE I. PHYSICO-CHEMICAL PROPERTIES OF THE SOIL  
(0–15 cm) AT THE NIAOULI RESEARCH STATION

Sand ———	Silt (%)	Clay ———	pH (KCl)	Org. C (%)	Total N (%)	C:N ratio	Bray I P (mg/kg)
89	4	7	4.4	0.37	0.041	9.02	0.70



The above-ground biomass was cut 16 weeks after planting, and all residues including the surface litter were air-dried and stored for the trial in 1998. The samples of the mucuna residues analysed by mass spectrometry gave an average of 3.12% N and 2.73%  $^{15}\text{N}$ -atom excess for the labelled mucuna and 3.32% N for the unenriched mucuna.

The experiment had a randomized complete block design with four replicates. The treatments included: (i) the control without fertilizer; (ii) 90 kg N ha<sup>-1</sup> of labelled  $^{15}\text{N}$  ammonium sulphate; (iii) 90 kg N ha<sup>-1</sup> of mucuna residue enriched in  $^{15}\text{N}$ ; (iv) 45 kg N ha<sup>-1</sup> of mucuna residue enriched in  $^{15}\text{N}$  combined to 45 kg N ha<sup>-1</sup> of unlabelled ammonium sulphate; (v) 45 kg N ha<sup>-1</sup> of unenriched mucuna residue combined to 45 kg N ha<sup>-1</sup> of  $^{15}\text{N}$  ammonium sulphate; (vi) 45 kg N ha<sup>-1</sup> of labelled  $^{15}\text{N}$  ammonium sulphate combined to 45 kg N ha<sup>-1</sup> of unlabelled ammonium sulphate; (vii) 45 kg N ha<sup>-1</sup> of mucuna residue enriched in  $^{15}\text{N}$  combined to the same rate of unenriched mucuna residue. The labelled ammonium sulphate used had an abundance of 10.16%  $^{15}\text{N}$ .

Each plot measured 3×2 m with a 2-m spacing between plots and 2.5 m between replications. Within the plots that received labelled N, a confined microplot (1.5×1.6 m) was marked out using metal roofing sheets 20-cm deep and 5 cm above, and was left bare during the experimental period. The roofing sheets were arranged in such a way that the maize roots could not go outside the microplot, while other roots (maize and weeds) were prevented from entering.

During preparations for planting, 7.2 kg DM of  $^{15}\text{N}$ -labelled mucuna residue, for the 90 kg N treatment, and 3.6 kg DM of unlabelled or labelled mucuna residue treatments, for the 45 kg N ha<sup>-1</sup>, were incorporated along the sowing lines to depth of 15 cm into the soil according to the treatment structure. Split applications of ammonium sulphate were made at 2 and 5 weeks after planting (WAP) maize. The 45 and 90 kg N ha<sup>-1</sup> treatments received respectively, 22.5 kg N ha<sup>-1</sup> and 45 kg N ha<sup>-1</sup> at each time application. Unlabelled or labelled N (ammonium sulphate) was dissolved and the solution was judiciously used to water each microplot. A uniform rate of 30 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, as ordinary superphosphate, was applied to all plots by incorporation along the sowing lines at emergence of the maize.

On 12 July 1998, maize cv. DMR (90 days) was hand-planted with a spacing of 80×20 cm, and thinned to one plant per hill (62,500 plants ha<sup>-1</sup>).

### 2.3. Plant sampling

Maize parts (shoot, cob and grain) were harvested and dry weights recorded after drying at 65°C for 72 h. The samples were pulverized with a ball mill and analyzed for total N and  $^{15}\text{N}$  enrichment by continuous-flow mass spectrometry, using a Roboprep C/N analyzer (Europa Scientific, Crewe, UK) interfaced with a Micromass 622 mass spectrometer (VG Isotopes Ltd., Cheshire, UK).

### 2.4. Calculations of percentage recovery

The percentage of recovery of N input (mineral or organic applied alone or in combination) was evaluated as follows.

(i) By the isotope method the percentage recovery of N applied to the soil is expressed as a quantitative measure of the N yield in the plant part in. This is shown in equation (1):

$$\% \text{ recov. of input N} = \frac{(\text{Amount of N in the plant part}) \times (\% \text{Ndf input in the plant part})}{(\text{Amount applied as fertilizer N})} \times 100$$

(ii) by the difference method, the percentage recovery of input N is expressed as a quantitative measure of the actual uptake by the plant minus the amount in the control in relation to the amount added to the soil. This is shown in equation (2):

$$\% \text{ utiliz' n of fert. N} = \frac{(\text{Plant N derived from fertilizer}) - (\text{Amount from the control})}{(\text{Total amount of N applied})} \times 100$$

## 2.5. Data analysis

The field data were examine by analysis of variance, using the SAS procedure [16]. To compare treatment means, the least significant difference (LSD) at the 0.05 level was utilized.

## 3. RESULTS

### 3.1. Maize dry matter yields and total N

Treatments that received 90 kg of labelled N as mucuna residues and those in which 45 kg N ha<sup>-1</sup> as residues were combined with the same rate of ammonium sulphate had total maize DM and N yields significantly higher ( $P < 0.05$ ) than with 90 kg of fertilizer N applied in a single dose (Table II).

The treatments composed of 90 kg N ha<sup>-1</sup> of labelled <sup>15</sup>N ammonium sulphate (F90\*) and 45 kg N ha<sup>-1</sup> of labelled <sup>15</sup>N ammonium sulphate combined to 45 kg N ha<sup>-1</sup> of unlabelled ammonium sulphate (F45\* F45) showed an average decrease in maize yield of 31% as compared to the overall average for the other treatments. The highest maize and N yields were obtained with the treatments 90 kg N ha<sup>-1</sup> of labelled mucuna residue (M90\*) and 45 kg N ha<sup>-1</sup> of labelled mucuna residue combined with 45 kg N ha<sup>-1</sup> of unlabelled ammonium sulphate (M45\* F45), which averaged 4,716 kg DM and 60 kg N ha<sup>-1</sup> (Table II).

### 3.2. Recovery of applied N

Total recoveries of labelled residue N and fertilizer N are given in Table III. Values obtained using the <sup>15</sup>N method were lower than those using the difference method. However, pool substitution was found to reduce the residue effect through immobilization of the organic input and, consequently, the %N recovery by the isotope method. In this situation, treatments where 45 kg N of ammonium sulphate were combined with 45 kg N ha<sup>-1</sup> of mucuna residue showed the highest value of % recovery of input. Similar trends were found with the difference method for the same treatments (Table III).

TABLE II. TOTAL DRY MATTER AND N YIELDS OF MAIZE GROWN AT NIAOULI IN 1998

Treatment	Total <sup>a</sup> DM yield (kg ha <sup>-1</sup> )	Total N yield (kg N ha <sup>-1</sup> )
F90*	2,887	42
M90*	4,716	61
M45* F45	4,715	59
M45 F45*	4,082	51
F45* F45	3,146	43
M45* M45	3,866	48
LSD <sub>0.05</sub>	1,464	16

<sup>a</sup>Grain plus stover.

TABLE III. FRACTIONAL RECOVERY  
OF INPUT N IN MAIZE GROWN AT  
NIAOULI IN 1998

Treatment	<sup>15</sup> N method (%)	Difference method (%)
F90*	7.50	14.1
M90*	4.18	35.4
M45* F45	15.1	66.6
M45 F45*	14.1	49.0
F45* F45	16.1	29.7
M45* M45	8.89	42.0

The isotope method indicated significantly lower percentage recovery of N than did the difference method

#### 4. DISCUSSION

Previous studies of organic residues in soil showed that rate of accumulation of organic N with N-fertilizer alone was less than with residue incorporation because conditions for substrate decomposition are less favourable in the absence of residue and/or N was lost by NH<sub>3</sub> volatilization [17,18]. This specific aspect was discussed in previous work by Houngnandan et al. [14] carried out at the same site in the DS of Benin, which showed an average increase of 49% in total plant yields and 36% in total N accumulation when mucuna residues were present as compared to N-fertilizer alone. Similar results were reported by Azam [19], Amara et al. [20], and Ngoran et al. [6]: lower shoot biomass production with mineral fertilizer applied alone.

Several workers have described positive effects on mineralization and availability to plants of soil N with increasing rates of fertilizer N, due to enhancement of microbial activity. They compared this to a priming effect [21] or to an added-N interaction that can be apparent or real [22–24].

Other results from the present study (<sup>15</sup>N method) showed lower recovery of N from mucuna residue compared to fertilizer N when the residue was used alone (Table III) confirming results of Ngoran et al. [6]. However, in contrast to these findings, work by Ladd et al. [3] and Xu et al. [25], stressed that <sup>15</sup>N recovery from labelled residue was higher than that from labelled fertilizer in the soil receiving residue and inorganic N in combination or separately. It was suggested that pool substitution reduced the residue effect through immobilization of the organic N and consequently decreased %N recovery using the isotope method.

Vanlauwe et al. [26] showed that residue quality has a major impact on the dynamics of applied residue N in alley-cropping systems, and suggested that it could be an important factor in deciding which residue-supplying plant species to integrate into such cropping systems. The rate of release and availability of residue N to crops has been discussed in many studies in terms of residue quality (chemical composition of the organic material) and soil type. In the evaluation of N recovery from mucuna placed on the surface or incorporated, Costa et al. [18] showed that when mucuna was incorporated, the net inorganic N accumulation was 60% higher after 178 days than when it was placed on the surface; moreover, significant amounts of N applied as surface-placed legume mulch were not found in the remaining undecomposed residue or as inorganic N in the soil. The authors observed that after 3 and 178 days, 15 and 45% of the applied N was not recovered, respectively. These data and other literature suggest that part of this unrecovered N was lost by ammonia volatilization. This point should be considered in further studies by assessing possible interactions between residue N and fertilizer N including the quality of mucuna residues and the soil type.

## ACKNOWLEDGEMENTS

The authors gratefully acknowledge the RAMR project (for financial support), funding by the Royal Tropical Institute (KIT) in Amsterdam, and the International Atomic Energy Agency, Seibersdorf, for analysing the samples. Our thanks to the Belgian Administration for Development Cooperation (BADC), and the International Institute for Tropical Agriculture, Ibadan, Nigeria, for providing a Graduate Research Fellowship.

## REFERENCES

- [1] KUMWENDA, J.D.T., et al., Soil Fertility Management Research on the Maize Cropping Systems of Smallholders in Southern Africa: A Review, NRG Paper 96-02, CIMMYT, Mexico D.F. (1996).
- [2] McCOWN, R.L., JONES, R.K., "Agriculture of semi-arid eastern Kenya: problems and possibilities", Sustainable Dryland Cropping (PROBERT, M.E., Ed.), ACIAR Proceedings No. 41, ACIAR, Canberra (1992) 8–14.
- [3] LADD, J.N., et al., Distribution and recovery of nitrogen from legume residues decomposing in soils sown to wheat in the field, *Soil Biol. Biochem.* **13** (1981) 251–256.
- [4] MULLER, M.M., SUNDMAN, V., The fate of nitrogen ( $^{15}\text{N}$ ) released from different plants materials during decomposition under field conditions, *Plant Soil* **105** (1988) 133–139.
- [5] HAGGAR, J.P., et al., Nitrogen dynamics of tropical agroforestry and annual cropping systems, *Soil Biol. Biochem.* **25** (1993) 1363–1378.
- [6] NGORAN, K., et al., Availability of N from *Casuarina* residues and inorganic N to maize using  $^{15}\text{N}$  isotope techniques, *Biol. Fert. Soils* **28** (1998) 95–100.
- [7] VANLAUWE, B., et al., Soil litter dynamics and N use in a leucaena [*Leucaena leucocephala* Lam. (De Witt)] alley cropping system in south-western Nigeria, *Soil Biol. Biochem.* **28** (1996) 739–749.
- [8] GILLER, K.E., WILSON, K.J., Nitrogen Fixation in Tropical Cropping Systems, CAB International, Wallingford (1991).
- [9] GILLER, K.E., et al., "Potential benefits from interactions between mineral and organic nutrients sources", Soil Fertility Research for Maize-Based Farming Systems in Malawi and Zimbabwe, Soil Fertility Network and CIMMYT-Zimbabwe, Harare, Zimbabwe (1998) 155–158.
- [10] PALM, C.A., et al., "Organic matter management: from science to practice", Soil Fertility Research for Maize-Based Farming Systems in Malawi and Zimbabwe, Soil Fertility Network and CIMMYT-Zimbabwe, Harare, Zimbabwe (1998) 21–29.
- [11] PALM, C.A., SANCHEZ, P.A., Nitrogen release from leaves of some tropical legumes as affected by their lignin and polyphenolic contents, *Soil Biol. Biochem.* **23** (1991) 83–88.
- [12] TIAN, G., et al., Biological effects of plant residues with contrasting chemical compositions under humid tropical conditions-decomposition and nutrient release, *Soil Biol. Biochem.* **24** (1992) 1051–1060.
- [13] IWUAFOR, E.N.O., et al., "On-farm evaluation of the contribution of sole and mixed applications of organic matter and urea to maize grain production in the savanna", Integrated Soil Fertility Management in Sub-Saharan Africa: From Concept to Practice (VANLAUWE, B., et al., Eds.) CAB International, Wallingford (2001) in press.
- [14] HOUNGNANDAN, P., Effect of mucuna residue management on nitrogen use by maize in the derived savannah, southern Benin Republic, *Field Crops Res.* (submitted).
- [15] FAO, World Soil Resources: an Explanatory Note on the FAO World Resources Map at 1:25,000,000 scale, Food and Agricultural Organization of the United Nations, Rome (1991).
- [16] SAS Institute Inc., SAS/STAT User's Guide, Version 6, 4<sup>th</sup> Edition, Vol. 2, SAS Institute, Inc., Cary, NC (1990).
- [17] JANZEN, H.H., MCGINN, S.M. Volatile loss of nitrogen during decomposition of legume green manure, *Soil Biol. Biochem.* **23** (1991) 291–297.
- [18] COSTA, F.J.S.A., et al., Evaluation of N recovery from mucuna placed on the surface or incorporated in a Brazilian oxisol, *Plant Soil* **124** (1990) 91–96.

- [19] AZAM, F., Comparative effects of organic and inorganic nitrogen sources applied to a flooded soil on rice yield and availability of N, *Plant Soil* **125** (1990) 255–262.
- [20] AMARA, D.S., et al., (1994) Uptake of N from fertilizer and prunings of *Gliricidia sepium*, International Atomic Energy (IAEA) Technical Document SM-334/14, Vienna (1994) 321–326.
- [21] WESTERMAN, R.L., KURTZ, L.T., Priming effects of <sup>15</sup>N-labelled fertilizers on soil nitrogen in field experiment, *Soil Sci. Soc. Am. Proc.* **37** (1973) 725–727.
- [22] JENKINSON, D.S., et al., Interactions between fertilizer nitrogen and soil nitrogen-the so-called ‘priming’ effect, *J. Soil Sci.* **36** (1985) 425–444.
- [23] RAO, A.C.S., et al., Influence of added nitrogen interactions in estimating recovery efficiency of labelled nitrogen, *Soil Sci. Soc. Am. J.* **55** (1991) 1616–1621.
- [24] AZAM, F., et al., Mineralization of N from plant residues and its interaction with native soil N. *Soil Biol. Biochem.* **25** (1993) 1787–1792.
- [25] XU, Z.H., et al., Nitrogen cycling in leucaena (*Leucaena leucocephala*) alley cropping in semi-arid tropics. II. Response of maize growth to addition of nitrogen fertilizer and plant residues, *Plant Soil* **148** (1993) 73–82.
- [26] VANLAUWE, B., et al., Recovery of *Leucaena* and *Dactyladenia* residue nitrogen-15 in alley cropping systems, *Soil Sci. Soc. Am. J.* **62** (1998) 454–460.

## EVALUATION OF AGRONOMIC EFFECTS OF ROCK PHOSPHATE USING RADIOISOTOPE TECHNIQUES\*

I. BOGDEVITCH\*\*, S. TARASIUK, Y. PUTYATIN, G. PIROGOVSKAYA, V. SOROKA  
Belarusian Research Institute for Soil Science and Agrochemistry (BRISSA),  
Minsk, Belarus

F. ZAPATA\*\*\*

Soil and Water Management & Crop Nutrition Section, Seibersdorf Laboratory,  
International Atomic Energy Agency, Vienna

### Abstract

The agronomic effects of rock phosphate (RP), in comparison with monoammonium phosphate (MAP), on limed sod-podzolic and peat soils were studied in pot experiments using the  $^{32}\text{P}$ -dilution technique. The effects of MAP application were greater than RP fertilizer. Beneficial effects of RP modified with an acidifying amendment were close to those of MAP. The root uptake of  $^{137}\text{Cs}$  by plants was lower with RP than with MAP. Taking into account the differences in costs of the fertilizers, RP application on acid peat soils ( $\text{pH}_{\text{H}_2\text{O}} < 5.0$ ) may have utility for improving the usefulness of radionuclide-contaminated grassland.

### 1. INTRODUCTION

Monoammonium phosphate (MAP) is one of the main types of water-soluble P fertilizers produced in Belarus. Like other water-soluble P fertilizers, it is expensive. During the period 1991–95 its application decreased from 194,000 to 27,000 tons of P, resulting in decreased agricultural productivity in Belarus. Now there is evidence that poor P balance in agricultural soils will have long-term negative effects on fertility.

There is an urgent need to find alternative sources of P to replace expensive water-soluble fertilizers. Direct application of rock phosphate (RP), one possible way to solve this problem, was widely practised in Belarus in the 1960s. Experiments (using  $^{32}\text{P}$ ) at that time showed that crop responses to RP in acid soils with low reserves of P were larger than in limed soils with better P status [1–3]. After intensive programs of liming of acid soils were initiated in Belarus, RP consumption for direct application rapidly decreased. During the past 20 years, RP has not been used as a P fertilizer, although it has been used for the synthesis of compound fertilizers.

There are significant deposits of RP in Belarus. Therefore, we undertook a programme of research to study the possibility of profitable application of finely powdered RP, which is significantly cheaper than locally produced water-soluble P fertilizers. The direct application of RP might be effective on acid (or even moderately limed) sod-podzolic and peat soils, which occupy about 30% of agricultural land of Belarus, and part of which was contaminated by radionuclides from the Chernobyl accident.

With the goal of increasing the agronomic effectiveness of RP fertilizer on the mineral limed soils, the effects of acidifying amendments were also examined.

---

\*Work performed within the framework of the IAEA Coordinated Research Project on The Use of Nuclear and Related Techniques for Evaluating the Agronomic Effectiveness of P Fertilizers, In Particular Rock Phosphate; RC No 9447.

\*\*Present address: Belarusian Research Institute for Soil Science and Agrochemistry, Kazintsa str. 62, 220108 Minsk, Belarus.

\*\*\*Present address: Soil and Water Management & Crop Nutrition Section, IAEA, Wagramer Strasse 5, P.O. Box 100, A-1400 Vienna, Austria.

TABLE I. SELECTED PROPERTIES OF THE SOD-PODZOLIC AND PEAT SOILS

Parameter	Method	Sod-podzolic	Peat
pH (H <sub>2</sub> O)	1:2.5–25 (soil : water) <sup>a</sup>	6.20	4.90
(pH (KCl)	1:2.5–25 (soil : KCl)	5.00	4.15
Organic matter (%)	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> oxidation	1.34	—
Extractable P (mg/kg)	0.2 M HCl	67.0	30.3
Available P (mg/L)	0.01 M CaCl <sub>2</sub>	0.14	0.12
Cation exchange capacity [cmol(+)/kg]	Buffer solution BaCl <sub>2</sub>	18.1	70.0
Exchangeable cations [cmol(+)/kg]			
Ca	1 M KCl	5.20	25.0
Mg	1 M KCl	1.30	12.8
K	1 M NH <sub>4</sub> OAc, pH 7.0	0.23	0.60
Texture (%)	Pipette method		
Clay		24.7	—
Silt		55.7	—
Sand		19.6	—

<sup>a</sup>Ratio soil: water, 1:25 with the peat soil and 1:2.5 with the podzolic soil.

The aims of these studies were:

- to determine the fractions of P in plants derived from RP and MAP using <sup>32</sup>P,
- to evaluate P availability from RP and MAP for lupine and ryegrass, and examine the effects of P-fertilizers on uptake of radionuclide <sup>137</sup>Cs in sod-podzolic and peat soils,
- to evaluate the possibility of direct application of cheaper powdered RP as an alternative to water-soluble P fertilizers,
- to assess the agronomic effects of modified RPs in comparison to MAP.

## 2. MATERIALS AND METHODS

### 2.1. Experiment A: isotope-dilution method

A rock phosphate from the Briansk deposit (Russia) was used in this study, containing 8.3% total P, 0.04% water-soluble P, and 2.9% citric acid-soluble P. MAP contains 21.8% total P and 20.4% water-soluble P.

Two soils were used, some properties of which are listed in Table I. The sod-podzolic silty clay loam soil is characterized by a medium levels of acidity and of extractable and available P. The peat soil is characterized by a higher level of acidity and lower levels of extractable and available P.

Evaluation of RP as a direct fertilizer in comparative with MAP was based on the <sup>32</sup>P-dilution technique [4].

A solution of NaH<sub>2</sub>PO<sub>4</sub> (4.4 mg P/kg soil), labelled with <sup>32</sup>P (3.7×10<sup>6</sup> Bq) was diluted in 200 mL of distilled water and applied to 5 kg of soil and thoroughly mixed. The soil was placed in plastic pots (5 kg/pot) and allowed to equilibrate for a week, after which the unlabelled fertilizers were applied to the sod-podzolic soil (1997) as follows:

- Control (22 mg N +140 mg K)/kg soil
- Rock phosphate (40 mg P + 22 mg N +140 mg K)/kg soil
- Monoammonium phosphate (40 mg P +140 mg K)/kg soil)

There was no need to apply additional N fertilizer to lupine. Therefore, the minimal rate of 22 mg N/kg soil was applied, equivalent to the N content of MAP.

With the peat soil (1998) the fertilizers were applied as follows:

- Control (80 mg N + 170 mg K)/kg soil
- Rock phosphate (40 mg P + 80 mg N + 170 mg K)/kg soil
- Monoammonium phosphate (40 mg P + 58 mg N + 170 mg K)/kg soil

There were four replications per treatment. Pregerminated seeds of yellow lupine (*Lupinus luteus*), cv. Pava, were sown in 1997 (twenty per pot), and seeds of ryegrass (*Lolium multiflorum* Lam. *westerwoldicum*), cv. Avante, in 1998 (thirty per pot; lupine is not recommended for cultivation on this peat soil). The pots were randomized and soil moisture was kept at 40 to 60% of field capacity. After two months growth, the shoots were harvested, dried at 65°C and weighed. Shoot P content was measured by the molybdate blue method. Two-gram samples were ashed at 450°C, dissolved in 20 mL of 1 M HCl and filtered. The  $^{32}\text{P}$  activity was measured by Cerenkov counting in a liquid scintillation counter. The amounts of P derived from soil and from tested fertilizers were calculated using the isotope-dilution procedure [6,7].

## 2.2. Experiment B: with $^{137}\text{Cs}$ contaminated soil

The effect of P fertilizers on the uptake of the radionuclide  $^{137}\text{Cs}$  was studied on the same soils in a pot experiment with parallel sets of the above-mentioned treatments. Both soils are typical for the area contaminated with  $^{137}\text{Cs}$  as a result of the Chernobyl accident.

The solution of  $\text{CsCl}_2$ , labelled with 900 Bq  $^{137}\text{Cs}$ , was diluted in 200 mL of distilled water per 1 kg of soil was and thoroughly mixed with the soil, which was placed in plastic pots (5 kg/pot) and allowed to equilibrate for a week. Then unlabelled fertilizers were applied according to the treatments described for Experiment A. The plants were harvested, cut into small pieces and dried at 65°C; determination of  $^{137}\text{Cs}$  activity was by gamma-spectrometry.

## 2.3. Experiment C: with modified RPs

This experiment (1999) was on a sod-podzolic silty clay loam soil, characterized by a medium level of acidity ( $\text{pH}_{\text{KCl}}$  6.0) and of extractable P (105 mg P/kg). The modified RPs had acidifying amendments, either of plant residues (organic, designated RPo) or with an inorganic chemical (RPc). To evaluate the effectiveness of modified RPs it was necessary to compare with MAP and RP without acidification. The P fertilizers were applied at 50 mg P/kg soil. An RP from the Mstislavl deposit (Belarus) was used (12.8% total P, 0.1% water soluble P, 8.6% citric-acid soluble P).

Soil mixed with fertilizers was placed in plastic pots. There were four replications per treatment. The seeds of oil radish (*Raphanus sativus* var. Oleifera) were planted (thirty seeds per pot) and the treatments randomized. Soil moisture was maintained at 40 to 60% of field capacity.

# 3. RESULTS AND DISCUSSION

## 3.1. Experiment A

The lupine and ryegrass plants treated with RP grew significantly more vigorously than did control plants, but less well than the MAP-treated plants (Table II). Supplied with RP and MAP, lupine shoots took up, respectively, 2.6 and 4.3 mg P/pot more than did plants with no P treatment. The dry weights of lupine shoots were also increased, respectively, by 9 and 15% as compared with the control. The effects of RP and MAP were more pronounced with ryegrass in the peat soil. The shoots took up 2.7 and 9.4 mg P/pot more than in the control plants.



In comparison to the treatment without P, specific activity (SA) of the plants decreased as a result of extra P from RP and MAP (Table III). For lupine, the Pdff values, i.e. the fractions of P in the plants derived from the applied RP and MAP were similar at 7.4 and 8.4%, respectively. P fertilizer-recovery values for RP and MAP were only about 1% because of the relatively high content of available native soil P. Similar results were found for the  $A_L$  values. It may be concluded that sod-podzolic soil has an availability of 500 mg P as RP-equivalent and 436 mg P as MAP-equivalent units. It is evident that, for lupine plants, 1 kg P as MAP is equivalent of 1.15 kg of RP ( $500/436 = 1.15$ ). Probably high relative availability of RP comparatively with MAP (RAID = 88%) was due to a greater ability of lupine to utilize P from insoluble forms.

For ryegrass on the peat soil, the Pdff values were 15% of RP and 22% of MAP. P fertilizer recovery values for RP and MAP were 1.5 and 2.9%, respectively, as a consequence of lower reserves of P. The  $A_L$  value for MAP (141 mg P/kg) was much lower than for RP (229). The higher P-uptake efficiency of ryegrass from MAP was due to its water-soluble P component. Therefore, for ryegrass, 1 kg P as MAP was equivalent to 1.62 kg of RP. The application of MAP had stronger beneficial effects than did RP (RAID = 67%) on this soil. However, the application of RP on the peat soil, which had a lower level of the native P fertility, provided a statistically significant increase of ryegrass shoot yield of 6% over the control.

The bioavailable P from the soil and P fertilizers used in Experiment A were determined by the isotopic exchange kinetics method [5]. It was found that the fixing capacity of soil ( $r_1/R$ ) remained low to medium after a 40-day period of incubation in wet conditions at temperatures of 25 to 28°C (Table IV). The  $C_p$  value (intensity factor) of the control treatment was slightly lower than 0.2 mg/L and the level of directly available P ( $E_1$ ) was medium at 4.4 mg P/kg, therefore, P was not the chief factor limiting plant growth.

After application of RP at 50 mg P/kg soil, the directly available P ( $E_1$ ) and other more-mobile pools, as A, B, and C were not increased (Table V), whereas by applying MAP, the available P content of the soil was significantly increased. As the fixing capacity was low to medium, a large part of the P applied as MAP remained directly available. The kinetic exchange constants indicated that mobility of the P ions was not significantly increased by RP application.

TABLE II. SHOOT DRY WEIGHT OF LUPINE AND RYEGRASS AND P UPTAKE (EXPT. A)

Treatment	Shoot dry weight (g/pot)	P concentration (%)	Total P uptake (mg P/pot)
Lupine (sod-podzolic soil)			
Without P	12.8	0.188	24.1
RP	14.0	0.191	26.7
MAP	14.7	0.193	28.4
LSD <sub>0.05</sub>	0.3	0.01	0.7
Ryegrass (peat soil)			
Without P	15.4	0.110	16.9
RP	16.3	0.120	19.6
MAP	18.8	0.140	26.3
LSD <sub>0.05</sub>	0.6	0.01	0.9

TABLE III. SPECIFIC ACTIVITY OF LUPINE AND RYEGRASS AND FERTILIZER-P UPTAKE PARAMETERS (EXPT. A)

Treatment	Specific activities (Bq/mg P)	Pdf soil (%)	Pdff (%)	P fert. recov. (%)	A <sub>L</sub> value (mg P/kg)
Lupine (sod-podzolic soil)					
Without P	22.9	100	—	—	—
RP	21.2	93	7.4	1.0	500
MAP	20.9	92	8.4	1.2	436
Ryegrass (peat soil)					
Without P	73.4	100	—	—	—
RP	62.5	85	15	1.5	229
MAP	57.2	78	22	2.9	141

TABLE IV. SOIL P PARAMETERS (EXPT. A)

Treatment	pH (H <sub>2</sub> O)	C <sub>p</sub> (mg P/L)	r <sub>1</sub> /R	n	E <sub>1</sub> (mg P/kg)	E <sub>1</sub> /C <sub>p</sub> (L/kg)	Total P (mg P/kg)
Without P	6.2	0.15	0.34	0.30	4.4	29.3	473
RP	6.2	0.20	0.36	0.25	5.5	27.5	523
MAP	6.0	1.10	0.54	0.14	20.0	18.2	523

The effect of RP and MAP on soil fertility properties after harvesting the crops is shown in Table VI. There was no significant difference between pH values for the controls without P and the treatments with MAP and RP. It is known that most of the native P in soil is fixed in unavailable forms. Also fertilizer P becomes converted in soils with time. Even the amounts classified as “available” by most chemical soil-testing methods are not directly soluble and available to plants. For example, it could be concluded that P fertility of the sod-podzolic soil was significantly increased to similar levels after RP and MAP application, when tested using the standard Belarusian method (0.2 M HCl extraction). But there was only a little increase in available P in 0.01 M CaCl<sub>2</sub> solution after RP application, whereas after MAP application there was a three-fold increase of P content.

Thus, it may be seen that the CaCl<sub>2</sub> extraction method was able to detect differences in available P in sod-podzolic soil amended with RP and MAP relatively close to the <sup>32</sup>P-exchange kinetic method of Dr. Fardeau (Table IV). The local standard method (0.2 M HCl) overestimated the effect of natural RP on soil-available P.

TABLE V. COMPARATIVE ANALYSIS AND KINETIC PARAMETERS OF SOIL P (AFTER AN INCUBATION PERIOD OF 40 DAYS) (EXPT. A)

Treatment	P pools (mg P/kg soil)				K <sub>m</sub> (min <sup>-1</sup> )	T <sub>m</sub> (min·10 <sup>-2</sup> )	F <sub>m</sub> (mg min <sup>-1</sup> )
	A	B	C	D			
Without P	32	78	50	319	11.0	9	16.4
RP	27	56	28	407	15.0	7	30.0
MAP	31	38	15	420	11.0	9	125

TABLE VI. EFFECT OF FERTILIZERS ON AVAILABLE P IN SOD-PODZOLIC AND PEAT SOILS EVALUATED BY CHEMICAL METHODS (EXPT. A)

Treatment	pH (KCl)	Extractable P (0.2 M HCl) (ppm)	Available P (0.01 M CaCl <sub>2</sub> ) (mg/L)
Sod-podzolic soil			
Without P	5.00	64	0.14
RP	5.20	101	0.19
MAP	4.90	111	0.43
LSD <sub>0.05</sub>	0.20	16	0.09
Peat soil			
Without P	4.15	30	0.12
RP	4.20	62	0.23
MAP	4.15	59	0.27
LSD <sub>0.05</sub>	0.20	2	0.03

The peat soil P fertility was also significantly increased, to approximately the same levels after application of RP and MAP. The determination of P fertility by 0.2 M HCl method and 0.01 M CaCl<sub>2</sub> extracting method did not show significant superiority, as in the previous method, for peat soil.

Therefore, P applications on sod-podzolic soils with medium P availability have to be mainly for soil fertility maintenance in rates close to the P output by harvested yields. For most of crops, water-soluble P forms, such as MAP, are preferred. But for plants with a high ability to utilize P, such as lupine, RP may be used for direct application as well as water-soluble P fertilizers on acid soils (pH<sub>H2O</sub> <6.0, pH<sub>KCl</sub> <5.0).

The effect of water-soluble P fertilizer, MAP, on ryegrass was stronger than that of RP on acid peat soil, in spite of the comparatively low level of the native soil P fertility. But, cost of fertilizer must be taken into consideration. One ton of P in the form of MAP on the Belarusian market was US\$773, whereas 1 ton of P as RP was US\$481. Therefore, the direct application of finely powdered RP on acid peat soil (pH<sub>H2O</sub> <5.0), especially for radical improvement of grassland on radionuclide-contaminated soil, may be feasible.

### 3.3. Experiment B

It is known that P and K fertilizers applied to radioactive contaminated soil may significantly decrease radionuclide uptake by plants, as well as increase yields. Therefore, the effects of RP and MAP on uptake of <sup>137</sup>Cs were studied on these two soils with a parallel pot experiment.

The yields were close to those obtained in Experiment A (Table VII). Significant reductions were seen in uptake of <sup>137</sup>Cs by lupine with RP (–16%) and MAP (–8%) in comparison with the plant activity of the control [8]. The activity decrease in the ryegrass on the peat soil was 27% with RP and only 7% with MAP. The stronger effect of RP may be explained by the effect of its higher C content on root activity.

The results suggest that direct application of RP may be more effective than water-soluble P fertilizers in reducing the plant uptake of <sup>137</sup>Cs on acid sod-podzolic and peat soils. These data are important, because the tested soils are common in the radioactivity-contaminated area.

TABLE VII. THE INFLUENCE OF P FERTILIZERS ON  $^{137}\text{Cs}$  CONTENT OF LUPINE SHOOTS ON SOD-PODZOLIC SOIL AND OF RYEGRASS ON PEAT SOIL (EXPT. B)

Treatment	Shoot dry weight (g/pot)	$^{137}\text{Cs}$ activity of shoots (Bq/kg)
Lupine		
Without P	13.0	109
RP	14.1	91
MAP	14.5	101
LSD <sub>0.05</sub>	0.5	5.0
Ryegrass		
Without P	15.1	1156
RP	16.3	847
MAP	18.7	1084
LSD <sub>0.05</sub>	0.5	65

### 3.4. Experiment C

Modified RPs more effectively increased shoot growth than did unmodified RP (Fig. 1.). The highest shoot dry weight of oil radish was obtained with MAP (18.9 g/pot). Shoot dry weights with the modified RPs were not significantly different (16.6–17.6 g/pot). The shoot yield was increased by 21 to 28% in comparison with RP treatment without acidifying amendment (13.7 g/pot).

Thus, these preliminary data show that the development of new ways of acidifying RP may be important to increase the crop yields at levels close those with MAP. This research will be continued.

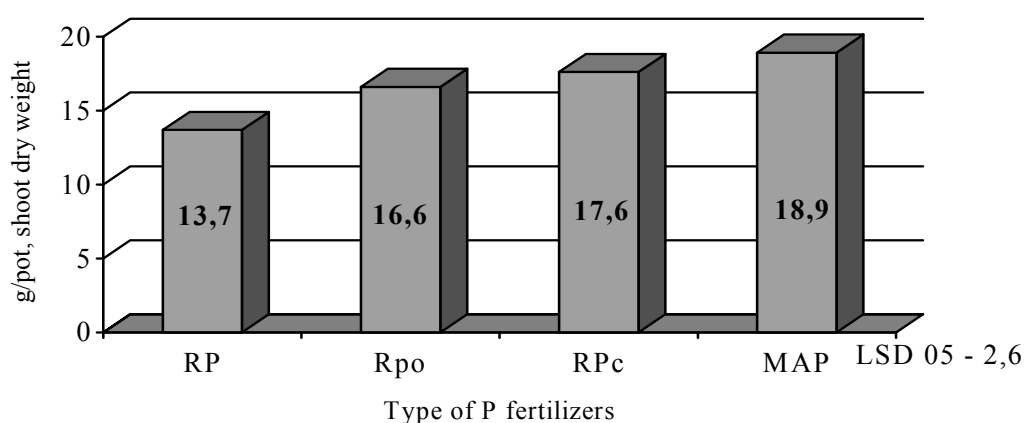


FIG.1. The influence of modified RPs on shoot yield of oil radish in comparison with unmodified RP and MAP (Expt. C).

#### 4. CONCLUSIONS

- The application of RP and MAP made similar moderate contributions to P nutrition of lupine grown on moderately limed sod-podzolic silty clay loam soil with  $\text{pH}_{\text{H}_2\text{O}}$  6.2,  $\text{pH}_{\text{KCl}}$  5.0 and a medium level of available P.
- The effect of MAP on ryegrass growth on the acid peat soil ( $\text{pH}_{\text{H}_2\text{O}}$  4.9,  $\text{pH}_{\text{KCl}}$  4.1) was much stronger than that of RP, with a comparatively low level of soil P fertility.
- The direct application of finely powdered RP on acid peat soil ( $\text{pH}_{\text{H}_2\text{O}} < 5.0$ ), especially for radical improvement of grassland on the radionuclide-contaminated area may be reasonable if fertilizer costs are taken into consideration.
- The positive effects of RP modified with acidifying amendments were close to the effect of MAP on limed sod-podzolic silty clay loam soil.

#### ACKNOWLEDGEMENTS

The research team sincerely appreciates the assistance and encouragement of colleagues at IAEA. Thanks are also to Dr. J.-C. Fardeau (CEA CADARACHE) for soil analysis, valuable comments and recommendations. Financial support of the IAEA and the French Government is gratefully acknowledged.

#### REFERENCES

- [1] SIROTIN, Y.P., "Problems of rock phosphate use in agriculture of USSR", Perspectives of Fertilizer Assortment Expansion, MSU, Moscow (1987) 39–41.
- [2] IVANOV, S.N., KRUGLOV, L.V., Comparative study of P availability from superphosphate and rock phosphate to fodder beans by means of  $^{32}\text{P}$  techniques, Rep. Belarusian Acad. Sci. (Minsk) **10** (1966) 50–52.
- [3] SEMENENKO, N.N., Efficiency of superphosphate and rock phosphate on potato on peat soils, Soil Sci. Agrochem. (Minsk) **10** (1973) 130–137.
- [4] ZAPATA, F., "Isotope techniques in soil fertility and plant nutrition study", Use of Nuclear Techniques in Studies of Soil-Plant Relationships, Training Course Series No. 2 (HARDARSON, G., Ed.), IAEA, Vienna (1990) 27–61.
- [5] FARDEAU, J.-C., et al., "Bioavailable soil P as a key to sustainable agriculture," Nuclear Techniques in Soil-Plant Studies for Sustainable Agriculture and Environmental Preservation, IAEA, Vienna (1995) 131–144.
- [6] ZAPATA, F., AXMANN, H.,  $^{32}\text{P}$  isotopic techniques for evaluating the agronomic effectiveness of rock phosphate materials, Fert. Res. **41** (1995) 189–195.
- [7] ZAPATA, F., The Agronomic Evaluation of Rock Phosphates Using Radioisotope Techniques, Experimental Guidelines, IAEA, Vienna (1984).
- [8] BOGDEVITCH, I.M., et al., "Effect of phosphorus fertilizers on  $^{137}\text{Cs}$  accumulation of crops", Soil – Fertilizer – Fertility (BOGDEVITCH, I., Ed.), Proc. International Scientific-Practical Conf. 16–19 February, Minsk, BAA, Gorki (1999) 170–172.

## USING $^{32}\text{P}$ METHODOLOGY TO ELUCIDATE ROOT DISTRIBUTION AND COMPETITION FOR NUTRIENTS IN INTERCROPPED PLANT COMMUNITIES

H. HAUGGAARD-NIELSEN, P. AMBUS  
Plant Biology and Biochemistry Department,  
Risø National Laboratory,  
Roskilde, Denmark

E.S. JENSEN  
Department of Agricultural Sciences,  
The Royal Veterinary and Agricultural University,  
Agrovetvej, Denmark

Intercropping involves the simultaneous cultivation of more than one plant species in the same field. This cropping strategy is known to improve the use of plant-growth resources in space and time. Technical difficulties in determining belowground competition complicate improvements in the understanding and management of competitive interactions among species. The present work evaluates a method (modified from [1]) for the study of root distribution.

A field study was carried out in 1999 at Risø National Laboratory, Denmark (55°41'N, 12°05'E), on a sandy loam with 11% clay. Field pea and spring barley were grown as sole crops and intercrops using a replacement design. The experimental plots (6×3.4 m) were laid out in a complete one-factorial randomized design including three replicates. In the lab, 0.45 mL  $^{32}\text{PO}_4$  solution (0.22 mCi mL<sup>-1</sup>) were dispensed into gelatine capsules arranged in copper trays placed over dry ice. In four microplots (50×50 cm) within each main plot, the frozen capsules were introduced to the soil via sixteen individual PVC-tubes (12-mm diam.) installed at four depths: 12.5, 37.5, 62.5 and 87.5 cm to simulate root distribution in the 0 to 25, 25 to 50, 50 to 75, and 75 to 100 cm soil layers, respectively. Holes were drilled using a 10-mm auger prior to installation of the PVC tubes by means of push rods fitted inside the tubes. After introducing the capsules, the tubes were filled and compressed with washed sand. The tubes were kept in the soil throughout the experiment. The second highest fully developed leaves from twenty-five individual plants in each microplot were collected for each sampling. Samples were taken continuously from 25 days after germination. Measured radioactivity (cpm mg<sup>-1</sup> dry leaf biomass) was used as a qualitative measure of root distribution by scintillation counting in Cerenkov mode. Sampling inside and in the rows next to the microplot represented vertical and horizontal root distributions, respectively.

Data show that about 95% of the vertical pea root system was confined to the upper 12.5 cm soil layer. In comparison, 25 to 30% of barley's vertical root system was distributed from 12.5 to 62.5 cm (Fig. 1). The barley root system was fully established in the 0 to 12.5 cm soil layer 25 days after germination, whereas this occurred 10 days later for pea (data not shown). Pea showed no differences in the rate of vertical root growth comparing sole cropping and intercropping whereas intercropped barley distributed a significantly higher proportion of the vertical root system from 37.5 to 62.5 cm compared to sole-cropped counterpart (Fig. 1). More rapid horizontal root development occurred in the intercrop than the sole crop for both species, and more rapidly for barley than for pea. However, late in the season, the horizontal root pattern was more ambiguous.

It was found that barley had faster root-system growth compared to pea, which is one possible explanation for barley being the stronger competitor in the intercrop. Another important finding was that intercropping compared to pure-stand cropping induced a deeper barley root system and faster horizontal root development by both species, indicating a potential improvement in the search for natural nutrient sources. Other data from the present study, using  $^{15}\text{N}$ , showed that it is possible to increase input of biological nitrogen fixation into temperate agroecosystems using pea-barley intercropping without compromising N-use efficiency, yield level or stability, as discussed Jensen [2].

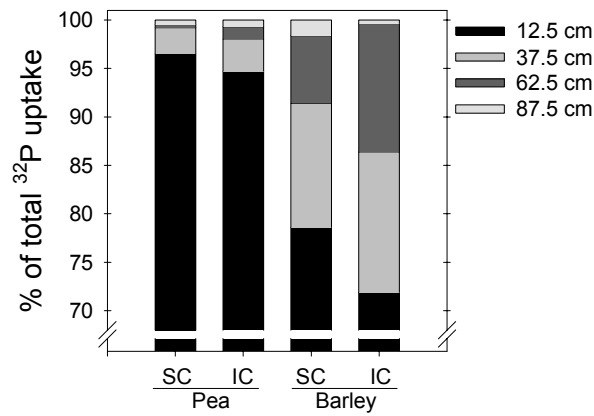


FIG. 1.  $^{32}\text{P}$  uptake from four depths: 12.5, 37.5, 62.5 and 87.5 cm as percentage of total  $^{32}\text{P}$  activity in harvested biomass. IC = intercropped and SC = sole cropped.

The modified method shows some obvious advantages:

- Preparation of the radioactive  $^{32}\text{P}$  solution/capsules is done in the laboratory where all appropriate precautions for contamination can be taken.
- Accurate amounts of radioactive tracers are precisely placed at specific soil depths without any contamination hazards for the soil layers around the deposition.
- It is easy to differentiate between root distribution of simultaneous growing intercropped plant species taking individual leaf samples.
- Compared to [1], this modified method minimizes changes of soil structure and compression of soil material caused by preparing an access hole for the capsules. In addition, the 2-mm difference between auger and PVC-tubes avoids air gaps along the soil-tube interface.

## REFERENCES

- [1] JACOBS, E., et al., A convenient method of placing radioactive substances in soil for studies of root development, *Agron. J.* **62** (1970) 303–304.
- [2] JENSEN, E.S., Grain yields, symbiotic  $\text{N}_2$ -fixation and interspecific competition for inorganic N in pea-barley intercrops, *Plant Soil* **182** (1996) 25–38.

## TRANSFER OF PHOSPHORUS IN SOIL-PLANT AND SOIL-SOLUTION SYSTEMS USING ISOTOPIC LABELING AND DILUTION METHODS

C. MOREL, A. SCHNEIDER

Institut National de la Recherche Agronomique (INRA)-Agronomie,  
Villenave d'Ornon

D. PLENET

INRA-Agronomie, Avignon

J.C. FARDEAU

INRA-Environnement et Agronomie, Versailles

France

### Abstract

A mechanistically-based characterization of plant-available soil P was obtained by labelling the isotopically exchangeable P and comparing, after similar periods, either the isotopic composition of P taken up by plants or the isotopic composition of P ions in soil suspensions (1 g:10 mL). The isotopic composition data did not differ significantly for different soil-solution-plant-fertilizer systems. As a consequence, plant-available soil P can be identified with the isotopically exchangeable P, i.e. soil P that has the same isotopic composition as P in solution. As a consequence, a deterministic modelling of P-ion transfer between soil and solution was developed using isotopic methods in soil suspensions for samples collected from a long-term field experiment conducted on P fertilization. The model, which is a kinetic Freundlich equation, accurately accounts for changes in the P concentration in solution and the periods of transfer. A laboratory procedure is proposed to quickly and easily determine the three-parameter estimates of the kinetic Freundlich equation. In this procedure, the range of P concentrations due to long-term P fertilization is mimicked by applying increasing rates of water-soluble P and by equilibrating these P-enriched soil suspensions before analyzing for P released to solution. The three-parameter estimates describing the soil-to-solution transfer of P ions were similar for all rates of laboratory-applied P. The modelling of the P-ion transfer is, therefore, independent of initial P concentration in solution. As a result, the time and soil-solution P dependence of the soil-to-solution transfer of P ions can be assessed by a simple and rapid laboratory procedure.

### 1. INTRODUCTION

Plant roots absorb phosphate (P) ions in solution. But, since only about 1% of the P taken up by roots is in solution, more or less 99% of it is derived from the soil solid phase. Therefore, the rate at which soil P is released to the solution is the main factor controlling P supply to plant roots and P-input efficiency. This transfer is also a major process involved in P release from sediments to surface waters.

The transfer of P ions within the soil-solution-root system depends on several kinetic, dynamic and interactive mechanisms governing the plant-availability of soil P. Reactions of adsorption-desorption, dissolution-precipitation, oxydo-reduction, complexation and mineralization control the P concentration in solution [1]. Also, a variety of mechanisms is developed by growing roots to acquire P [2] and environmental factors can strongly influence the degree of expression of a given mechanism. For example, soil-P level affects root activity since root exudates are generally released in greater quantities from plants deficient in P.

In the present study, we review data on the characterization of plant-available P, using isotopic labelling of isotopically exchangeable P, and highlight recent advances, obtained with this approach, on the modelling of plant-available soil P. The transfer of P ions between soil and solution for soil samples collected from a long-term field P experiment is accurately modelled. A laboratory procedure is proposed to quickly and easily determine the amount of P ions released from soil to solution as a function of soil solution P and time.



## 2. MECHANISTICALLY-BASED CHARACTERIZATION OF PLANT-AVAILABLE SOIL P

### 2.1. Comparison of isotopic composition of P taken up by different plant species

The isotopically exchangeable P, also called the E value, is by definition the amount of P in a soil that has the same isotopic composition (IC) as P ions in solution. It is an operationally defined component of soil P since it quantifies soil P that can replenish the solution. It identifies the fraction of P in the solid phase that is in dynamic equilibrium with the P ions in solution.

The origin of P taken up by various species of plants and the effects of mycorrhizal inoculation have been examined in many pot experiments. The principle consists, first, of isotopically labelling the exchanged P in the soil. In a second step, the radioactive soil is cultivated for a period of 2 to 3 months. The radioactivity and total P are determined to calculate the IC of the P taken up by plant, i.e. the radioactivity per unit of P assimilated. Then, the IC of P taken up is compared for different plant species after (or not) mycorrhizal inoculation [3,4]. For a given soil, identical IC values for P taken up by different plants indicate similar origins of the assimilated P. In contrast, significant differences in ICs indicate that a fraction of the assimilated P came from a different source. Although the P taken up by plants can vary greatly, the ICs of P taken up were similar for different plants when cultivated in the same  $^{32}\text{P}$ -labelled soil after they were (or not) inoculated by mycorrhizal fungi [3,4]. This suggests that, in many temperate agricultural soils, different plant species do not significantly mobilize specific sources of P in soils by producing root exudates, by altering rhizosphere pH, etc.

### 2.2. Comparison of plant isotopic composition of P and isotopic composition of P ions in solution

A further detailed analysis of the origin of plant-available P was obtained by comparing the IC of P taken up by plants and the IC of P ions in solution after similar periods [5].

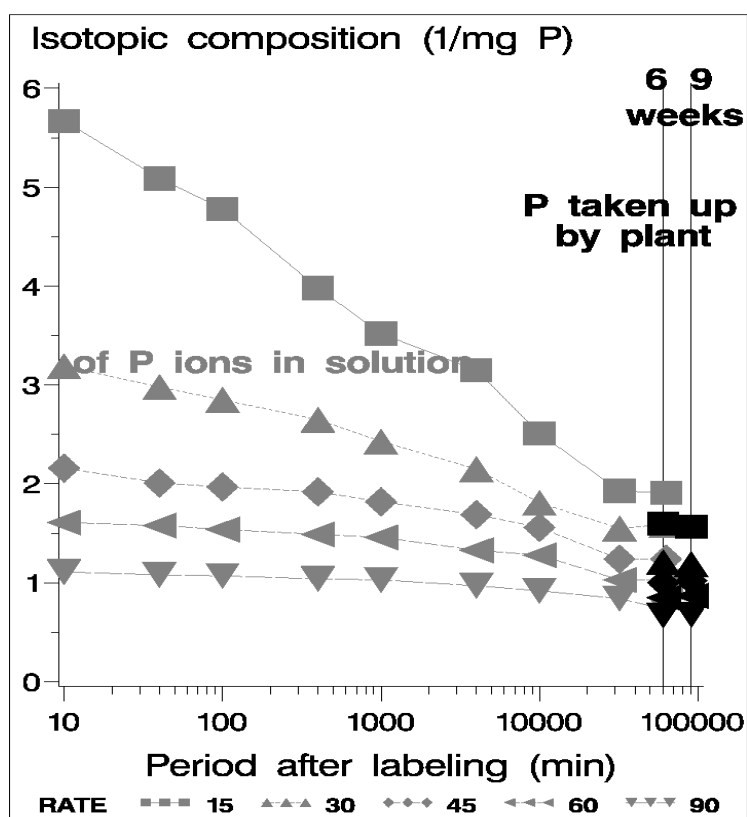


FIG. 1 Kinetics of the isotopic composition ( $\text{mg P}^{-1}$ ) of P ions in solution and P taken up by ryegrass after 6 and 9 weeks of cultivation after applying five rates of water-soluble P labelled with  $^{32}\text{P}$ .

Figure 1 presents the kinetics of the IC of P ions in soil suspension (1 g:10 mL) up to about 6 weeks after adding five rates of water-soluble labelled P. The ICs of P taken up by ryegrass in pot experiment conducted in parallel on the same soil with the same five rates of P application were determined after 6 and 9 weeks of cropping [4]. For all rates of P application and periods of absorption, there were no significant differences between the values of IC of P taken up by plants and those of IC of P ions in solution (Fig. 1).

A review of the data published on the comparison of IC values of P taken by plant and of P ions in solution is presented in Fig. 2. The soil-plant-fertilizer factors were plant species, mycorrhizal inoculation, soil type, freshly applied or residual P, and P form. All data were inside the 95% limit of confidence and a linear regression ( $y=0.002+1.00x$ ,  $r^2=0.97$  for fifty-one observations) described the relation between the two sets of IC data. The slope and the ordinate were not significantly different from 1 and 0, respectively. Thus, there was a 1:1 relation between IC data—the IC of P ions in solution was an accurate and reliable predictor of the IC of P in the plants. Although the amount of P ions in solution represents less than 1% of the amount of P taken up by the plant, the P taken up by the plant had the same isotopic composition as the P ions in solution. Therefore, it can be concluded that the P taken up by the plant was supplied from soil P that had the same IC as the P ions in solution, i.e. the E value. A corollary result is that the plant-available P assessed by the L value [6], i.e. the amount of P in soil with the same IC as the P taken up by the plants, was not significantly different from the E value [7]. Another corollary result is that the relative contribution of applied P to plant nutrition can be predicted by the relative change in E values [8].

In conclusion, the comparison of the IC of P taken up by plants for varying soil-plant-fertilizer systems in pots and field conditions [10] and the IC of P ions in solution indicates that the plant-available P is the isotopically exchangeable P. In consequence, it is appropriate and relevant to analyse and understand the variability of E values to develop models of plant-available P in soils.

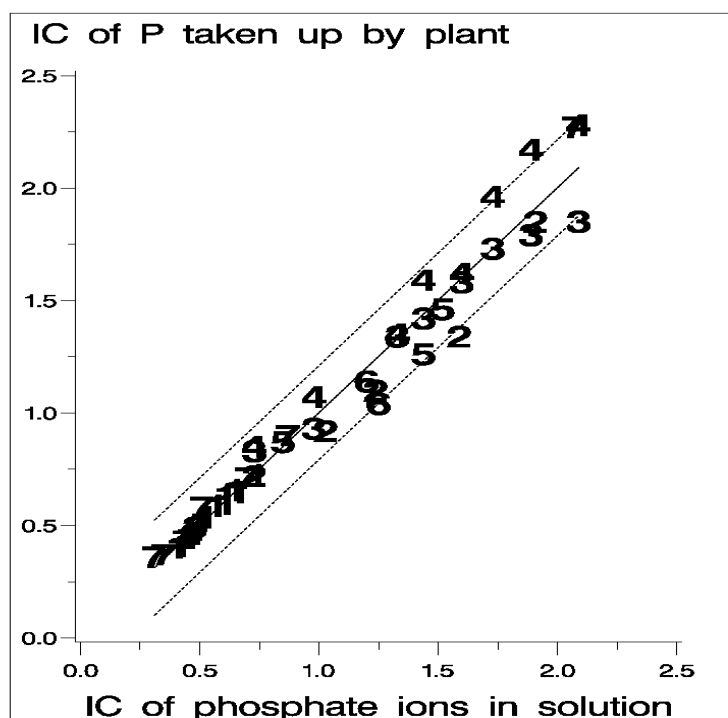


FIG. 2. Comparison between the isotopic composition (IC,  $10^{-2} \text{ mg P}^{-1}$ ) of P taken by plants and the IC of P ions in solution. Data were published as follows: 1 [5]; 2 and 6 [9]; 3 [4]; 4 and 5 [8]; 7 [7].

### 3. MODELLING PLANT-AVAILABLE P IN SOILS BY DESCRIBING THE TRANSFER OF P IONS BETWEEN SOIL AND SOLUTION

#### 3.1. Theory and calculations

The isotopic labelling of P ions in solution in soil suspensions is achieved by introducing a known amount of  $^{32}\text{P}$  or  $^{33}\text{P}$  as phosphate ions. The isotopic tracer is uniformly and instantaneously dispersed in the soil solution. The rate at which the isotopic tracer is diluted is the result both of the release rate of unlabelled P from soil to solution and of the removal rate of labelled P ions from solution to soil [11]. When  $^{32}\text{PO}_4$  (R) carrier-free solution [R is generally  $10^{-5}$  of the amount ( $Q_W$ ) of P ions in solution] is introduced in pre-equilibrated soil suspensions,  $Q_W$  remains constant with time for a few to several days, depending on soil type, P-fertilization history, and on microbial activity. In such a steady-state situation, the rate of P release is equal to the rate of P removal. Therefore, the amount of unlabelled soil P newly transferred to solution is determined by measuring both the isotopic composition (IC) of P ions in solution, i.e. labelled P ions per unit of total P ions, and  $Q_W$ . The total amount (E) of P ions in which R is diluted by isotopic exchange includes both  $Q_W$  and the amount ( $Q_r$ ) of P ions transferred from soil solid phase to solution. By definition, all E fractions have the same IC value, which gives:

$$\frac{R}{E} = \frac{r}{Q_W}$$

where

R/E is the IC of E,

r/ $Q_W$  is the IC ratio of  $Q_W$  and r, the radioactivity remaining in solution at time t.

E and  $Q_r$  are calculated as follows:

$$E = \frac{Q_W}{\left(\frac{r}{R}\right)} \text{ and}$$

$$Q_r = E - Q_W$$

$Q_W$ , R, and r are determined from  $^{31}\text{P}$  and  $^{32}\text{P}$  measurements made on aliquots of the solution. The radioactivities of r and R are counted within a few hours so that correction for decay is not necessary to calculate the isotopic dilution ratio r/R.

#### 3.2. Experimental determination and soil samples

Soil suspensions were prepared by adding 99 mL deionized water to 10 g of soil and pre-equilibrated for 16 h, stirring for 15 min/h. One mL of  $^{32}\text{PO}_4$  carrier-free (R) was introduced to soil suspensions. Fractions of soil suspension were taken up with a syringe after 1, 10, and 100 min, and filtered. P concentration ( $C_p$ ) in soil solution was determined colourimetrically. Radioactivity (R and r) was determined by liquid scintillation. Details of the protocol were reported by Fardeau (1996).

Soil samples were collected from a 17-year-old field experiment cropped continuously with maize. The soil is a loamy textured (53% silt) typical Luvisol from the southwest of France. The experimental design is a completely randomized block with four replicates. Soluble P fertilizer was applied as triple superphosphate (45%  $\text{P}_2\text{O}_5$ ) at four rates ( $\text{kg P ha}^{-1}$ ): 0 (P0), 26.2 every year (P1A), 52.4 every 2 years (P1B) and 78.6 every year (P3). All soil samples were air-dried and 2-mm sieved before P determination.

### 3.3. Results and Discussion

Typical experimental data depicting  $Q_T$  values after 1, 10 and 100 min are presented in the Fig. 3A for the sixteen soil samples (four blocks, four rates of P fertilization). The short-term kinetics data can be used to predict E values and, therefore,  $Q_T$  values, for periods of up to a few weeks [13,14].

Experimental  $Q_T$  values increased both with time (t) and with P concentration ion solution ( $C_P$ ). The following equation closely fitted the forty-eight experimental values:

$$Q_T = 4.84C_P^{0.694} t^{0.276}, r^2=0.99 \quad (1)$$

This description of the P ions released from soil to solution is a Freundlich's equation extended to all periods of transfer. This result is in agreement with previous published results in which periods of exchange were unique. For example, the curves of sorbed phosphate that was isotopically exchanged after 1 day were described by a Freundlich's equation in fourteen soils from Colombia and Brazil [15].

Equation (1) extended the validity of the Freundlich's equation to periods of from minutes to a few weeks. This extended kinetic Freundlich equation is similar to the description proposed by Barrow [16] and Chardon and Blaauw [17] of P-ion transfer between soil and solution assessed by sorption/desorption methods. Recent work [18] indicates that amounts of P transferred from soil to solution after various periods and for various initial soil-solution P levels do not differ significantly using either desorption by resin strips or the isotope-dilution method.

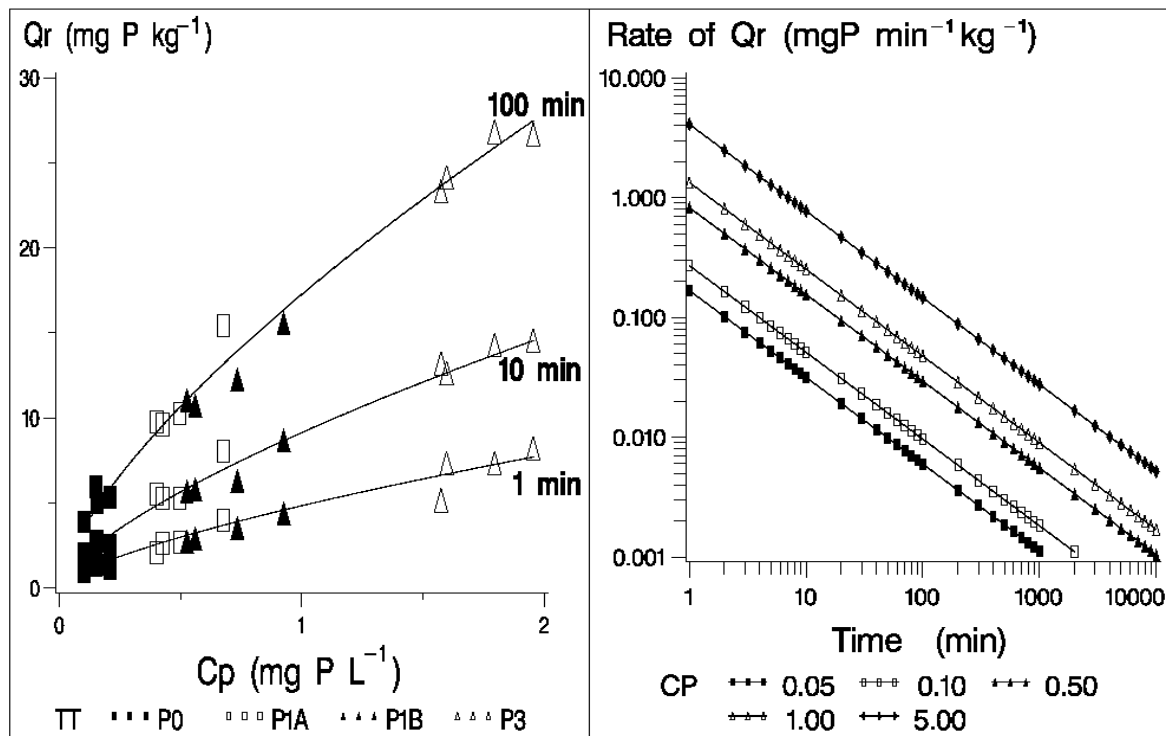


FIG. 3. A. Experimental and calculated amounts ( $Q_T$ ) of P ions transferred from soil to solution as a function of time and P concentration in solution ( $C_P$ ) in sixteen soil samples taken up from a 15-year-old field experiment. P0, P1A, P1B and P3 are fertilization treatments. B. Calculated values of the gross rate ( $dQ/dt$ ) of P-ion transfer from soil to solution for periods up to 10,000 min, i.e. about 1 week, and for P concentrations ( $C_P$ ) encountered in arable soils, i.e. 0.05 to 5 mg P L<sup>-1</sup>.

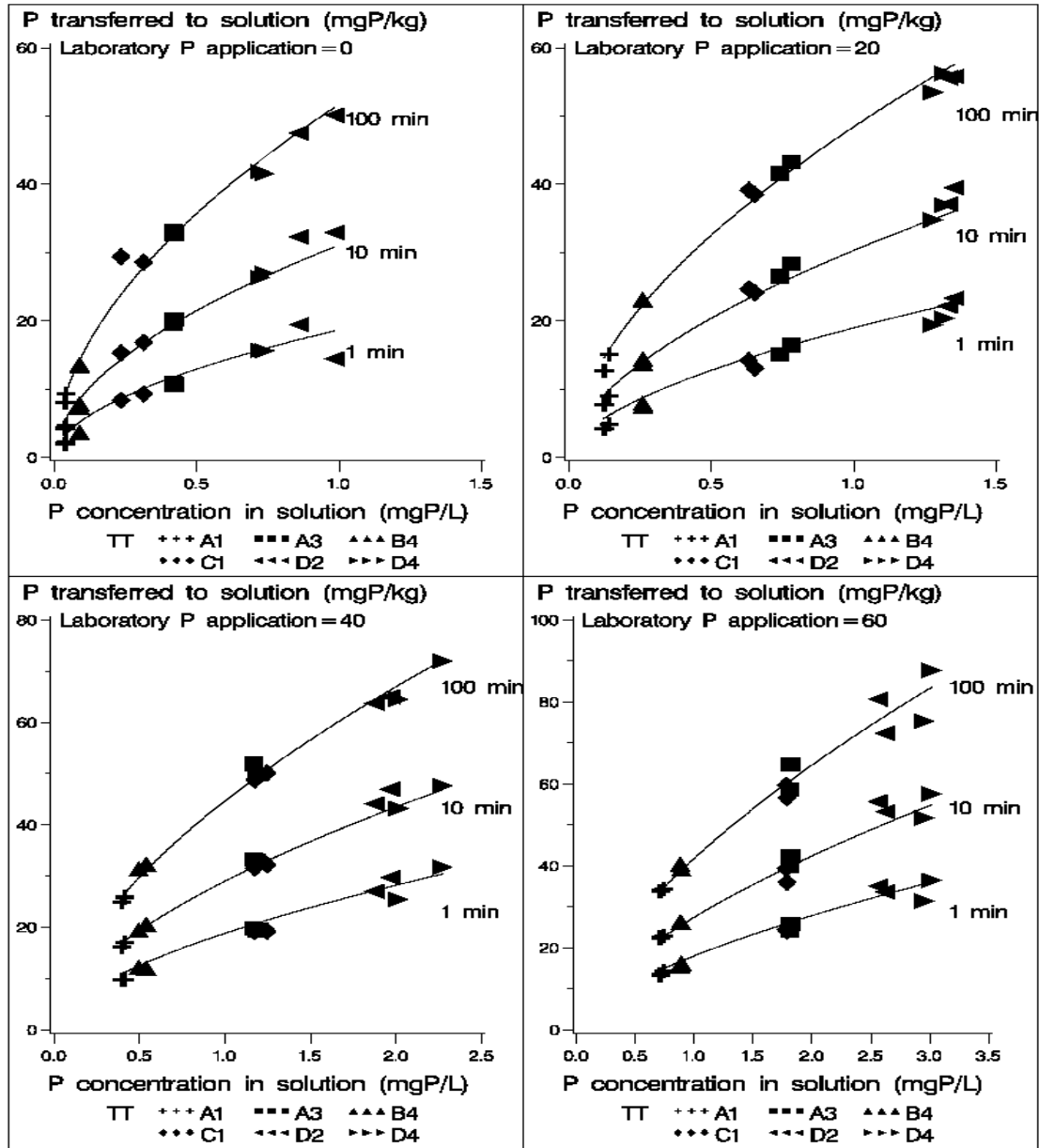


FIG. 4. Experimental and calculated amounts of P ions transferred from soil to solution as a function of time and P concentration in solution ( $C_P$ ) in six soil samples (two replicates) taken up from a 26-year field experiment. A1, A3, B4, C1, D2 and D4 are fertilization treatments applied during the 26-year period.

Assuming the steady-state condition, i.e.  $C_P$  remains constant with  $t$ , the gross rate of soil P released to solution,  $dQ_T/dt$  ( $\text{mg P kg}^{-1} \text{ min}^{-1}$ ), is calculated by the first-order derivative of eq. (1) to time:

$$\frac{dQ_T}{dt} = 1.34 \frac{C_P^{0.694}}{t^{0.724}} \quad (2)$$

The time-course of the  $dQ_T/dt$  values is depicted (Fig. 3B) in  $\log_{10}$ - $\log_{10}$  scales over 1 week for  $C_P$  levels encountered in temperate agricultural soils, i.e. 0.05, 0.1, 0.5, 1 and 5  $\text{mg P L}^{-1}$ . For a given  $C_P$  value,  $dQ_T/dt$  decreased with time whereas for a given time,  $dQ_T/dt$  increased with  $C_P$ . For instance, at  $C_P = 0.5 \text{ mg P L}^{-1}$ , the  $dQ_T/dt$  value at  $t = 1 \text{ min}$  was almost 800-fold higher than after 1 week. The ratio of  $dQ_T/dt$  at 5  $\text{mg P L}^{-1}$  to that at 0.05  $\text{mg P L}^{-1}$  was constant for all  $t$ .

TABLE I. PARAMETER ESTIMATES OF THE AMOUNT OF P TRANSFERRED BETWEEN SOIL AND SOLUTION AFTER ADDING 0, 20, 40 AND 60 mg P kg<sup>-1</sup> TO SIX SOIL SAMPLES COLLECTED FROM THE SAME FIELD EXPERIMENT, BUT WITH DIFFERENT P-FERTILIZATION HISTORIES. THE MODEL WAS  $Q_T = vC_P^W t^P$ , AND  $r^2$  IS THE PROPORTION OF VARIATION ACCOUNTED FOR BY THE MODEL.

P applied (mg P kg <sup>-1</sup> )	$v$	$W$	$P$	$r^2$	No. of observations
0	18.7 (0.7) <sup>a</sup>	0.53 (0.02)	0.221 (0.008)	0.98	35
20	18.9 (0.4)	0.57 (0.02)	0.203 (0.005)	>0.99	36
40	18.8 (0.3)	0.58 (0.01)	0.188 (0.004)	>0.99	36
60	18.0 (0.6)	0.63 (0.03)	0.183 (0.007)	0.98	36

<sup>a</sup>Asymptotic standard errors in parentheses.

The isotope-dilution method allows us to describe P-ion transfer between soil and solution as a function of  $t$  and  $C_P$ . It is rapid, simple, reliable and valid for  $C_P$  values encountered in arable soils and for periods relevant to P absorption by growing roots. Several operational results have already been obtained for agronomic purposes [14]. However, the evident limitation of this approach is the necessity to have available soil samples from a long-term field experiment.

The following study aimed to propose a simple and quick procedure to determine in the laboratory the kinetics of the Freundlich equation for transfer of P ions between soil and solution.

#### 4. MODELING OF PLANT-AVAILABLE P BY A LABORATORY PROCEDURE

Six soil samples, denoted A1, A3, B4, C1, D2 and D4, with various P histories were collected from the 26-year experiment. For all samples, 0, 20, 40 and 60 mg P kg<sup>-1</sup> were applied as KH<sub>2</sub>PO<sub>4</sub> to suspensions in the laboratory. The suspensions were equilibrated for 40 h and microbial activity was prevented by adding a biocide. Then, the  $Q_r$  values were determined as previously described.

The experimental and modelled values of  $Q_r$  are presented in Fig. 4 for the four rates of P application in the laboratory and the six soil samples. Parameter estimates of  $Q_T$  (mg P kg<sup>-1</sup>) as a function of soil solution P (mg P L<sup>-1</sup>) and time (min) are in Table I.

For all rates of added P, the parameter estimates describing the P ions released from soil to solution as a function of soil solution P and time were very close. This result was also observed in a long-term field experiment on P on a Canadian mollisol [19]. Therefore, it can be deduced that, the range of P concentrations obtained after adding P in laboratory studies and by equilibrating soil suspensions can mimic the range of P concentration in solution obtained by applying various P treatments in field experiments for long period of time.

#### 5. CONCLUSIONS

The measurement and comparison of the isotopic composition both of P taken up by plants and of P ions in soil-suspension solution, after similar periods of exchange, gave internal evidence that the isotopically exchanged P in soils is the plant-available P in many soil-plant-fertilizers systems. As a consequence, it is a relevant and reliable approach to analyse and describe the variability of the plant-available P in soils. The transfer of P ions between soil and solution can be easily monitored in soil suspensions by labelling P ions in solution and adopting isotope-dilution methods. After a long period of cultivation and P fertilization, the amount of P transferred between soil and solution depended on time and soil-solution P. The kinetic Freundlich's equation closely fitted experimental data for soil-solution P levels similar to those encountered in temperate agricultural soils and for periods relevant to absorption of P by roots. This equation can be determined by developing a simple and quick

laboratory procedure in which the ranges of soil-solution P levels are mimicked by applying increasing rates of P to soil suspensions, by equilibrating for a few hours and by determining the kinetics of P-ion transfer for all soil-solution P levels for short-term periods.

## REFERENCES

- [1] Frossard, E., et al., Processes governing phosphorus availability in temperate soils, *J. Env. Qual.* **29** (2000) 15–23.
- [2] Hinsinger, P., How do plant roots acquire P mineral nutrients? Chemical processes involved in the rhizosphere, *Adv. Agron.* **64** (1998) 225–266.
- [3] Bolan, N.S., A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants, *Plant Soil* **134** (1991) 189–207.
- [4] Morel, C., Plenchette, C., Is the isotopically exchangeable phosphate of a loamy soil the plant-available P? *Plant Soil* **158** (1994) 287–297.
- [5] Fardeau, J.C., Cinétiques de Dilution Isotopique et Phosphore Assimilable des Sols, PhD Thesis, University of Paris (1981).
- [6] Larsen, S., The use of  $^{32}\text{P}$  in studies on the uptake of phosphorus by plants, *Plant Soil* **4** (1952) 1–10.
- [7] Frossard, E., et al., Soil isotopically exchangeable phosphorus: a comparison between E and L values, *Soil Sci. Soc. Am. J.* **58** (1994) 846–851.
- [8] Morel C., Fardeau J.C., Phosphorus bioavailability of fertilizers: a predictive laboratory method for its evaluation, *Fert. Res.* **28** (1991) 1–9.
- [9] Morel C., Analyse par Traçage Isotopique du Comportement du Phosphore dans les Systèmes Sol-engrais-Plante: Conséquences en Matière de Fertilisation. PhD Thesis, University of Provence Aix-Marseille (1988).
- [10] Fardeau, J.C., Triboï, E., Mesure au champ du phosphore disponible ‘L’, *Plant Soil* **41** (1974) 293–302.
- [11] Di, H.J., et al., Isotopic dilution methods to determine the gross transformation rates of nitrogen, phosphorus, and sulfur in soil: a review of the theory, methodologies, and limitations, *Aust. J. Soil Res.*, **38** (2000) 213–230.
- [12] Fardeau, J.C., Dynamics of phosphate in soils. An isotopic outlook, *Fert. Res.* **45** (1996) 101–109.
- [13] Fardeau J.C., et al., Cinétique d’échange des ions phosphate dans les systèmes sol-solution. Vérification expérimentale de l’équation théorique, *CR Acad. Sci. Paris*, t 300, Sér. III, **8** (1985) 371–376.
- [14] Morel C., et al., Transfer of phosphate ions between soil and solution: perspectives in soil testing, *J. Env. Qual.* **29** (2000) 50–59.
- [15] Le Mare, P.H., Sorption of isotopically exchangeable and non-exchangeable phosphate by some soils of Colombia and Brazil, and comparisons with soils of southern Nigeria, *J. Soil Sci.*, **33** (1982) 691–707.
- [16] Barrow, N.J., The description of desorption of phosphate from soil, *J. Soil Sci.* **30** (1979) 259–270.
- [17] Chardon, W.J., Blaauw, D., Kinetic Freundlich equation applied to soils with a high residual phosphorus content, *Soil Sci.* **163** (1998) 30–35.
- [18] Schneider, A., Morel, C., Relationship between the isotopically exchangeable and resin-extractable phosphate of deficient to heavily fertilized soil, *Eur. J. Soil Sci.*, **51** (2000) 709–715.
- [19] Morel, C., Phosphorus transformation and availability under cropping and fertilization, *Soil Sci. Soc. Am. J.* **58** (1994) 1439–1445.

## ISOTOPIC STUDIES ON THE FERTILIZER VALUE OF SEWAGE SLUDGE FOR INCREASED CROP YIELD AND TO PRESERVE THE ENVIRONMENT

S. AHMED, S.M. RAHMAN, M.B. HOSSAIN  
Bangladesh Institute of Nuclear Agriculture,  
Mymensingh, Bangladesh

### Abstract

To investigate the effect on wheat yields of application of sewage sludge as sources of N and P, field experiments using  $^{15}\text{N}$  and a greenhouse experiment using  $^{32}\text{P}$  were conducted on a dark-grey floodplain soil (Haplaquept) of Bangladesh. Gamma-irradiation at 5 kGy reduced total bacterial counts and almost eliminated hazardous pathogenic microorganisms. When applied at the equivalent rate of  $400 \text{ kg N ha}^{-1}$ , sewage sludge produced wheat yields similar to those recorded with  $100 \text{ kg N ha}^{-1}$  (urea). The highest sludge-N recoveries were recorded with the highest application rate of  $400 \text{ kg N ha}^{-1}$  equivalent of sludge. The highest sludge-P recovery was obtained also with the  $400 \text{ kg N ha}^{-1}$  equivalent of irradiated sludge. Residual beneficial effects of sewage sludge application were observed in subsequently planted rice, whereas no similar effect was obtained after 3 years of application of  $100 \text{ kg N ha}^{-1}$  as urea. The  $^{15}\text{N}$  and  $^{32}\text{P}$  studies helped to quantify the amounts of N and P assimilated from sewage sludge and the degree of their utilization by wheat, thus confirming the potential value of sludge as fertilizer. For accurate estimation of N and P utilization, the isotope method was more reliable than the indirect method. Marginal improvement in soil fertility status and organic matter were observed. Heavy metal contents were quite low in this study indicating no immediate threat of contamination of soil and crops.

### 1. INTRODUCTION

Intensive farming for higher crop production has led to significant depletion of nutrients and losses of organic matter from soil due to “mining.” Integrated nutrient and fertilizer management practices, along with organic amendments, offer the opportunity to enrich soil and increase fertility and thereby maximize crop yields and sustain agricultural production. This has resulted in an increased interest for utilizing sewage sludge as an organic fertilizer.

With increases in urban populations, the safe disposal of sewage sludge is a growing problem that impinges on human and environmental health. On the other hand, sewage sludge is a nutrient-rich organic by-product of municipal wastewater treatment. Recycling of such wastes on agricultural land can provide a valuable source of essential plant nutrients and organic matter and may constitute an efficient method of disposal [1]. High levels of N, P and organic matter content in sewage sludge make it an excellent fertilizer and soil conditioner [2], and its application has been reported to enhance crop productivity [3]. Sewage sludge produced corn yields that were similar to, or better than, those obtained with an equal application of chemical N-fertilizer, and improved physical and biological properties of the soil [4].

Negative effects of sludge application result from the accumulation in soil of heavy metals and microorganisms that are pathogenic to humans and animals [5]. Risks from pathogens can be mitigated by pre-treatment through gamma-irradiation to kill microorganisms [6,7].

With proper soil-management practices, soil application of sewage sludge has the potential to be an ecologically and economically viable means of disposal. The advantages of using sewage sludge as fertilizer in agricultural soils may significantly outweigh the disadvantages.

The present paper reports results in the context of Bangladesh, generated under the auspices of a Joint FAO/IAEA Division's Co-ordinated Research Project: “The Use of Irradiated Sewage Sludge to Increase Soil Fertility, Crop Yields and to Preserve the Environment.”



## 2. MATERIALS AND METHODS

Field experiments using  $^{15}\text{N}$  and a greenhouse experiment using  $^{32}\text{P}$  were carried out at the experimental farm and greenhouse of the Bangladesh Institute of Nuclear Agriculture, Mymensingh. A dark-grey floodplain soil (Haplaquept), with a sandy-loam texture, was used. The moist sewage sludge, mainly domestic but partly industrial, was collected from a treatment plant of the Water and Sewerage Authority (WASA), Dhaka, and sun-dried for over a month. An adequate amount was ground, processed, and irradiated at 5 kGy in a  $^{60}\text{Co}$  gamma irradiator.

### 2.1. Field experiment

The field experiment comprised ten treatments:

- $T_1$ , 100 kg N  $\text{ha}^{-1}$  as urea,
- $T_2$ , 20 kg N  $\text{ha}^{-1}$  as urea,
- $T_3$ , non-irradiated sewage sludge (NIS) at 100 kg N  $\text{ha}^{-1}$  equivalent,
- $T_4$ , NIS at 200 kg N  $\text{ha}^{-1}$  equiv.,
- $T_5$ , NIS at 300 kg N  $\text{ha}^{-1}$  equiv.,
- $T_6$ , NIS at 400 kg N  $\text{ha}^{-1}$  equiv.,
- $T_7$ , irradiated (IRS) sewage sludge at 100 kg N  $\text{ha}^{-1}$  equiv.,
- $T_8$ , IRS at 200 kg N  $\text{ha}^{-1}$  equiv.,
- $T_9$ , IRS at 300 kg N  $\text{ha}^{-1}$  equiv.,
- $T_{10}$ , IRS at 400 N  $\text{ha}^{-1}$ .

Nitrogen-15-labelled urea was applied at 20 kg N  $\text{ha}^{-1}$  (10% a.e.) in treatments  $T_2$  to  $T_{10}$ . Unit plot size was 4×3 m of which 1×1m was delineated as the isotope sub-plot. The experimental hand an RCB design with four replications. The test crop was wheat (*Triticum aestivum* L. cv. Kanchan). It was harvested at maturity and total dry-matter yields recorded. Analyses for  $^{15}\text{N}$  and total N were done at IAEA's Soils Laboratory, Seibersdorf, Austria. Isotope-derived parameters were calculated using  $^{15}\text{N}$  data and the percent N derived from sewage sludge estimated [8]. Microbiological analyses of sewage sludge and soil were performed in the Environmental Microbiological Laboratory, Dhaka University, using standard methods [9,10].

The physico-chemical characteristics of the initial soil, NIS, and IRS are presented in Table I. Microbiological analyses of NIS and IRS, and of soils from sludge-treated plots after 4 years of experiments, were recorded. Residual effects of sewage sludge on yields of rice (*Oryza sativa* cv. Binashail) were examined after wheat and fallow.

### 2.2. Greenhouse experiment

A greenhouse experiment, with wheat as the test crop, on the contribution of P from the various N-equivalent rates of sewage sludge (as in field experiment) was carried out with 3 kg soil per pot labelled with 8.4  $\mu\text{Ci}$   $^{32}\text{P}$  carrier-free orthophosphate at a rate equivalent of 40 kg P  $\text{ha}^{-1}$ . There were ten treatments with four replications each. The treatments were:

- $T_1$ , no P,
- $T_2$ , 100 kg N  $\text{ha}^{-1}$  as urea + 40 kg P  $\text{ha}^{-1}$ ,
- $T_3$  to  $T_{10}$ , same as those for the field experiment plus 40 kg P  $\text{ha}^{-1}$ .

The experiment was laid out in RCBD.

Total P and  $^{32}\text{P}$  contents of the plants were determined colourimetrically and liquid scintillation counting, respectively. Phosphorus uptake from the sewage sludge was calculated using the  $^{32}\text{P}$ -dilution method [8].

Relative effects of sewage-sludge application on soil properties and heavy-metal concentrations in total dry matter yield of wheat grown on sludge-treated soil for 4 years were assessed.

TABLE I. CHARACTERISTICS OF INITIAL SOIL, NON-IRRADIATED (NIS) AND IRRADIATED (IRS) SEWAGE SLUDGE

Parameters	Soil	NIS	IRS
pH (1:5 soil/sludge:CaCl <sub>2</sub> )	6.7	4.7	4.6
Bulk density (g cm <sup>-3</sup> )	1.34	—	—
Water holding capacity (%)	45.2	—	—
CEC (meq%)	10.5	8.3	9.3
Organic matter (%)	1.2	12.0	12.2
Total N (%)	0.09	0.90	0.95
Bray II extractable P (mg kg <sup>-1</sup> )	9.3	496	431
Heavy metals:			
Aqua-Regia extract			
Zn (mg kg <sup>-1</sup> )	21.0	420	419
Cu (mg kg <sup>-1</sup> )	19.0	408	423
Pb (mg kg <sup>-1</sup> )	14.0	188	192
DTPA extract	0.25	1.50	1.30
Zn (mg kg <sup>-1</sup> )	9.0	334	340
Cu (mg kg <sup>-1</sup> )	8.2	110	99.0
Pb (mg kg <sup>-1</sup> )	2.0	39.0	41.6
Cd (mg kg <sup>-1</sup> )	BDL <sup>b</sup>	BDL	BDL

<sup>a</sup>Diethylthiamine pentaacetic acid. <sup>b</sup>Below detectable limit.

### 3. RESULTS AND DISCUSSION

#### 3.1. Microbiological analyses of sewage sludge and experimental soil

Gamma-irradiation of sewage sludge at 5 kGy proved effective in reducing total bacterial counts and eliminating potentially pathogenic coliforms (Table II).

#### 3.2. Wheat yield and N uptake

The highest dry-matter yields of wheat were obtained with sewage sludge at 400 kg N ha<sup>-1</sup> equivalent, comparable to that recorded with 100 kg N ha<sup>-1</sup> as urea (Table III). Enhanced crop yields as a result of application of irradiated sludge have been explained in terms of inactivation of growth inhibitors in sludge by irradiation [11]. Amongst our sludge treatments, the highest sludge N yield of 10.9 kg ha<sup>-1</sup> and percent N recovery of 34 by wheat were recorded in the treatment receiving the highest rate, 400 kg N equivalent ha<sup>-1</sup> of irradiated sludge, treatment T<sub>10</sub>. There was, however, no significant difference in percent N derived from sewage sludge (NdfSS) and sludge-N yield between NIS and IRS plots, which varied between 10 to 13.5% and 4.30 to 10.9 kg ha<sup>-1</sup> respectively, in different sludge treatments.

The highest grain yields of wheat were recorded in the third year, and were lowest in the first year (Fig. 1). The highest yield, obtained with 400 kg N equivalent ha<sup>-1</sup> of irradiated sludge (T<sub>10</sub>), was almost identical to that obtained with 100 kg N ha<sup>-1</sup> of urea (T<sub>1</sub>). Mineralization of nutrients and particularly of N from sludge appeared to be slow initially, but had a cumulative effect with time.

TABLE II. MICROBIOLOGICAL ANALYSIS OF  
NON-IRRADIATED (NIS), IRRADIATED (IRS) SEWAGE  
SLUDGE AND SLUDGE-AMENDED SOIL

Parameter	NIS	IRS	Soil + NIS	Soil + IRS
	(g <sup>-1</sup> DM)			
Total bacteria (37°C)	1.2×10 <sup>6</sup>	4.0×10 <sup>3</sup>	1.5×10 <sup>5</sup>	1.1×10 <sup>2</sup>
Total coliforms (37°C)	3.0×10 <sup>4</sup>	Nil	1.7×10 <sup>2</sup>	<1
Faecal coliforms	1.0×10 <sup>2</sup>	Nil	<1	Nil
<i>E. coli</i>	5.6×10 <sup>3</sup>	Nil	1.5×10 <sup>2</sup>	<1
<i>Salmonella</i>	Nil	Nil	0.8×10 <sup>2</sup>	Nil
<i>Ascaris ova</i>	Nil	Nil	Nil	Nil

TABLE III. TREATMENT EFFECTS ON WHEAT YIELD AND N UPTAKE

Treatment	Total yield (kg DM ha <sup>-1</sup> )	N yield (kg ha <sup>-1</sup> )	Ndff (%)	Fert. N yield (kg ha <sup>-1</sup> )	NdfSS <sup>a</sup> (%)	Sludge N yield (kg ha <sup>-1</sup> )	N recov. (%)
T <sub>1</sub>	7,116	78.3	66	52.0	—	—	52
T <sub>2</sub>	2,400	24.9	8.2	2.05	—	—	10
T <sub>3</sub>	3,608	41.8	6.3	2.63	10	4.30	13
T <sub>4</sub>	4,609	50.3	8.2	4.11	12	5.91	21
T <sub>5</sub>	5,635	62.6	8.5	5.33	13	4.48	27
T <sub>6</sub>	6,989	76.1	7.5	5.73	13	9.93	29
T <sub>7</sub>	4,004	39.8	8.1	3.22	12	4.68	16
T <sub>8</sub>	4,461	50.4	7.8	3.94	12	5.89	20
T <sub>9</sub>	6,004	68.0	7.6	5.19	12	8.34	26
T <sub>10</sub>	7,126	82.5	8.3	6.87	13	10.9	34
LSD <sub>0.05</sub>	244	8.4	4.9	—	NS	1.4	—

<sup>a</sup>N derived from sewage sludge.

### 3.3. Residual effects on rice

Residual beneficial effects of sludge application on rice yield (4,045 kg ha<sup>-1</sup>) were observed with 400 kg N equivalent ha<sup>-1</sup> of irradiated sewage sludge in the third crop of the rotation (Table IV). The yield was much higher than those produced after applications of chemical N fertilizer. The similarity of the yields with T<sub>1</sub> and T<sub>2</sub> indicate no residual effect from urea application. Similar long-term benefits from sewage-sludge application have been reported before [4].

### 3.4. Wheat yield and P uptake

In the pot experiment, treatments T<sub>6</sub> and T<sub>10</sub> (400 kg N equivalent of NIS and IRS, respectively) produced the highest dry-matter yields (14.2, 13.6 g pot<sup>-1</sup>, respectively) and of P (22.1, 24.3 mg pot<sup>-1</sup>, respectively) (Table V). Fertilizer-P yield was higher in T<sub>2</sub>, which received the recommended rate of 100 kg N ha<sup>-1</sup> (urea) and 40 kg ha<sup>-1</sup> labelled <sup>32</sup>P orthophosphate. Percent P derived from sewage sludge (PdfSS) ranged between 37 and 44; the highest value, obtained with T<sub>10</sub> receiving 400 kg N equivalent ha<sup>-1</sup> of irradiated sludge, was not significantly different from the others. The sludge-P yield and percent-P recovery were highest also in T<sub>10</sub>, compared to the other sludge treatments. Irradiated sewage sludge contributed more towards the P-pool.

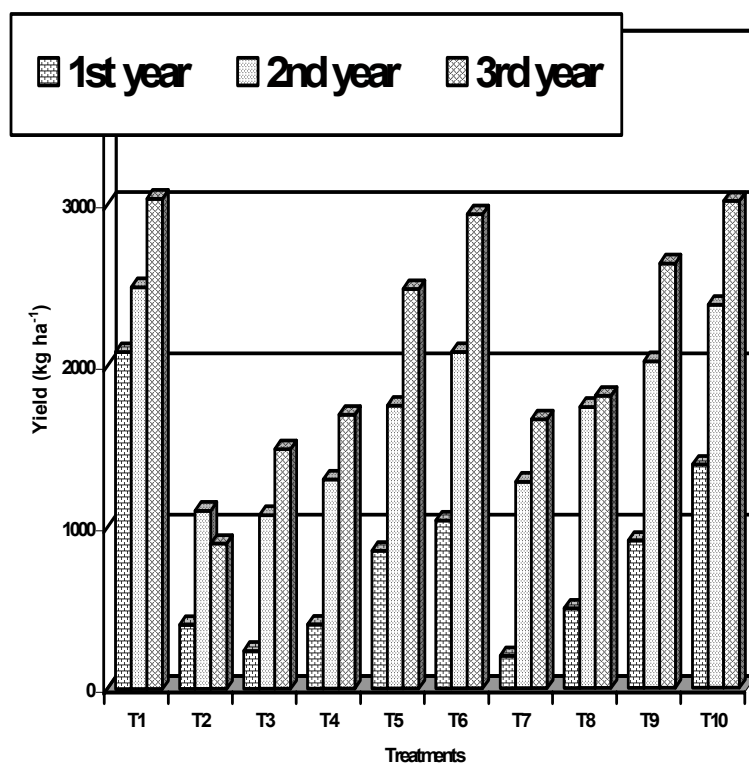


FIG.1. Grain yield of wheat with various treatments of non-irradiated and irradiated sewage sludge (using  $^{15}\text{N}$ ).

TABLE IV. RESIDUAL EFFECTS OF SEWAGE SLUDGE ON THE YIELD OF RICE

Treatment	Grain yield (kg ha <sup>-1</sup> )	Straw yield (kg ha <sup>-1</sup> )
T <sub>1</sub>	2,638	3,712
T <sub>2</sub>	2,505	3,540
T <sub>3</sub>	2,904	4,045
T <sub>4</sub>	3,140	4,499
T <sub>5</sub>	3,453	4,973
T <sub>6</sub>	4,017	5,240
T <sub>7</sub>	2,688	3,786
T <sub>8</sub>	3,218	4,589
T <sub>9</sub>	3,534	5,277
T <sub>10</sub>	4,045	5,362

The use of  $^{15}\text{N}$  and  $^{32}\text{P}$  helped to quantify the amounts of N and P from the sewage sludge recovered by wheat. The data confirmed the potential value of sewage sludge as a fertilizer. Isotope techniques are considered reliable for precise estimations of availability and utilization of nutrients by crops [8].

### 3.5. Effect on soil physico-chemical properties

Changes in soil fertility status after 4 years of sewage sludge application resulted in marginal improvements in some physical properties including organic matter, N and P content (compare Table VI with Table I). However, other research indicated that soil type plays a role, hence multi-location field trials are needed [4].

### 3.6. Effect on heavy-metal content in crop and soil

After 4 years of study, heavy-metal concentrations in wheat grown on sludge-treated soil were low and indicated no immediate concern of contamination as recorded in crops and soils (Table VII). Heavy metals are strongly retained in soil, and, as such, their soil-solution concentrations are usually low. Hence, heavy-metal losses by crop removal and leaching usually are small [12,13].

TABLE V. TREATMENT EFFECTS ON WHEAT YIELD AND P UPTAKE

Treatment	Total dry-matter (g pot <sup>-1</sup> )	Total P yield (mg pot <sup>-1</sup> )	Pdff (%)	Fert. P yield (mg pot <sup>-1</sup> )	PdfSS <sup>a</sup> (%)	Sludge P yield (mg pot <sup>-1</sup> )	P recov. (%)
T <sub>1</sub>	9.6	13.9	—	—	—	—	—
T <sub>2</sub>	10.1	15.9	15	2.4	—	—	4.1
T <sub>3</sub>	12.5	20.0	8.6	1.7	44	8.8	2.9
T <sub>4</sub>	13.0	21.0	8.9	1.9	40	8.4	3.1
T <sub>5</sub>	12.3	19.9	8.8	1.7	43	8.3	2.9
T <sub>6</sub>	14.2	22.1	9.7	2.1	37	8.3	3.5
T <sub>7</sub>	11.6	17.1	8.9	1.6	42	7.6	2.6
T <sub>8</sub>	13.6	21.8	8.6	1.9	40	8.8	3.1
T <sub>9</sub>	12.4	19.5	9.0	1.8	42	8.1	2.9
T <sub>10</sub>	13.6	24.3	9.2	2.2	44	10.8	3.7
LSD <sub>0.05</sub>	2.3	5.3	1.1	0.46	NS	1.2	0.78

<sup>a</sup>P derived from sewage sludge.

TABLE VI. RELATIVE EFFECTS OF SEWAGE SLUDGE APPLICATION ON PHYSICO-CHEMICAL PROPERTIES OF THE EXPERIMENTAL SOIL

Soil parameter	Value
pH(1:5 Soil:CaCl <sub>2</sub> )	6.7
Bulk density (g cm <sup>-3</sup> )	1.40
Water-holding capacity (%)	47.5
CEC (meq%)	12.2
Organic matter (%)	1.6
Total N (%)	0.12
Bray II extractable P (mg kg <sup>-1</sup> )	11.8

TABLE VII. HEAVY-METAL CONTENT IN WHEAT  
GROWN ON SLUDGE-AMENDED SOIL AND IN THE SOIL

Wheat/Soil	Treatments	Zn	Cu	Cd	Pb	Ni
		———— (mg kg <sup>-1</sup> ) ————				
Wheat	Control	38.8	3.9	0.12	0.17	1.2
	NIS treated	49.9	9.8	0.24	0.75	1.40
	IRS treated	50.9	8.8	0.22	0.71	1.37
Soil	Control plot	5.4	4.4	0.53	0.68	0.66
	NIS treated	17.0	10.1	0.21	1.32	2.1
	IRS treated	16.9	8.6	0.19	1.80	1.6

#### 4. CONCLUSIONS

With appropriate management practices, sewage sludge can be utilized as a soil conditioner and partial substitute for chemical N and P fertilizers, thus mitigating environmental pollution. Irrigated sludge is preferable in view of reduction in microorganisms possibly pathogenic to humans and animals.

#### ACKNOWLEDGEMENTS

The authors are grateful to the joint FAO/IAEA Division, of International Atomic Energy Agency, Vienna, for financial support and for <sup>15</sup>N analysis. We thankfully acknowledge Dr. P.M. Chalk, Head, Soil and Water Management & Crop Nutrition Section, for valuable guidelines and cooperation.

#### REFERENCES

- [1] KUMAZAWA, K., "Use of sewage sludge for agriculture in Japan", Sewage Sludge and Wastewater for Use in Agriculture, IAEA-TECDOC-971, Vienna (1997) 111–127.
- [2] SOMMERS, L.E., Chemical composition of sewage sludges and analysis of their potential use as fertilizer, J. Environ. Qual. **6** (1977) 225–232.
- [3] HERNANDEZ, T., et al., Influence of sewage sludge application on crop yields and heavy metal availability, Soil Sci. Plant Nutr. **37** (1991) 201–210.
- [4] SUSS, A., "Use of sewage sludge as a fertilizer for increasing soil fertility and crop production", Sewage Sludge and Wastewater for Use in Agriculture, IAEA-TECDOC-971, Vienna (1997) 129–137.
- [5] McGRATH, S.P., et al., Land application of sewage sludge: scientific perspectives of heavy metal loading limits in Europe and United States, Environ. Rev. **2** (1994) 109–117.
- [6] CHANG, A.C., "Land application of sewage sludge: Pathogen issues", Sewage Sludge and Wastewater for Use in Agriculture, IAEA-TECDOC-971, Vienna (1997) 183–190.
- [7] PIKAEV, A.K., "Current status of radiation treatment of water and wastewater", Sewage Sludge and Wastewater for Use in Agriculture, IAEA-TECDOC-971, Vienna (1997) 9–27.
- [8] HARDARSON, G., DANSO, S.K.A., "Use of <sup>15</sup>N methodology to assess biological nitrogen fixation", Use of Nuclear Techniques in Studies of Soil-Plant-Relationships. A Training Manual (HARDARSON, G., Ed.), IAEA, Vienna (1990) 129–160.
- [9] GARCIA, L.S., ASH, L.R., Diagnostic Parasitology, The C.V. Mosby Co., St. Louis (1975).
- [10] ANONYMOUS, Standard Methods for the Examination of Water and Wastewater (18th Ed.), American Public Health Assoc., New York (1992).
- [11] PANDEY, G.A., et al., Effect of gamma irradiated sludge on the growth and yield of rice (*Oryza sativa*, L. GR-3), Environ. Poll. **51** (1988) 563–573.
- [12] McGRATH, S.P., Long-Term Studies of Metal Following Application of Sewage Sludge in Pollutant Transport and Fate in Ecosystems, Bull. Ecol. Soc. SP. Public 6 (1987).
- [13] DOWDY, R.H., et al., Trace metal movement in an Aerie Ochraqualf following 14 years of annual sludge application, J. Environ. Qual. **20** (1991) 119–123.

# NITROGEN AND PHOSPHORUS FERTILIZATION OF RICE (*Oryza sativa* L.) GROWN IN AN ACID-SULPHATE SOIL OF THE MEKONG DELTA, VIET NAM

THU TRA LUONG, LE DAC LIEU

Radiobiology Department,  
Center for Nuclear Techniques

VAN TAM HOANG, AN CUONG PHAM

Soil Science Department,  
Institute of Agricultural Sciences of Southern Vietnam

Ho Chi Minh City, Viet Nam

## Abstract

A field experiment was carried out in Cuchi District, during the 1998 winter-spring season using the rice cultivar IR66. The fertilizer treatments consisted of a factorial combination of four N rates (0, 60, 90, 120 kg N ha<sup>-1</sup>) and three P rates (0, 60, 120 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>). The <sup>15</sup>N-labelled fertilizer used was urea, at 1.5% atom <sup>15</sup>N excess and the P fertilizer was Longthanh superphosphate, 16% P<sub>2</sub>O<sub>5</sub>. Nitrogen and P fertilization significantly increased total dry matter yield of rice over the no-fertilizer control. For the N fertilization, the highest grain yield was obtained with the 60 kg N ha<sup>-1</sup>, and it thereafter decreased with 90 and 120 kg N ha<sup>-1</sup>, whereas straw yields were increased at all N-applications rates. For P fertilization, straw and grain-yield increases were obtained at the highest rate of 120 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. Straw and grain total N increased with each rate of fertilizer N. Significant increases in %Ndff values for straw and grain were observed. Fertilizer-N recovery by the rice ranged from 26 to 32%, the highest value being for the 90 kg N ha<sup>-1</sup> rate. Fertilizer-N recovery by the rice was the lowest (27%) for the without-P control and increased with P fertilization up to 32% with 120 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. The results indicate that the optimum N and P fertilizer rates were determined as 90 kg N ha<sup>-1</sup> and 120 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> for rice grown on Cuchi acid-sulphate soil.

## 1. INTRODUCTION

In the Mekong Delta, the most important rice-producing area of Viet Nam, infertile acid sulphate soil occupies more than 60% of land (approximately 1,347,950 ha). Rice yields in this area are one third to a half of those obtained in alluvial soils [1]. The main soil constraints to crop production are: very high acidity (pH 3–4) associated low available P, low levels of organic matter, and toxicity of Al<sup>3+</sup> and Fe<sup>3+</sup>. For intensive rice production in these acid-sulphate soils, inputs of N and P are required [2].

Commercial sources of N and P are expensive for farmers, and there is need to gather information on crop-yield response to foster the efficient use of fertilizers. However, there is little quantitative information on the fate and efficiency of use of N and P fertilizers applied to rice grown in these acid soils. Therefore, the objectives of this study were : i) to evaluate rice responses to increasing N and P fertilization rates, and ii) to study the effects of N and P rates on the recovery of <sup>15</sup>N-labelled fertilizer.

## 2. MATERIALS AND METHODS

A field experiment was carried out at Cuchi District, during the 1998 winter-spring season using the rice cultivar IR66, which has a growth season of 100 to 105 days. The acid sulphate soil had the following properties, pH<sub>(H2O)</sub> 4.13; total N 0.383%; total P 0.116% P<sub>2</sub>O<sub>5</sub>; P available (Bray II) 4.3 ppm, total K 0.49% K<sub>2</sub>O; organic matter content 4.59%; CEC 8.32 cmol kg<sup>-1</sup>; Fe<sup>3+</sup> 2.61 cmol(+)kg<sup>-1</sup>.

The fertilizer treatments consisted of a factorial combination of four N rates (0, 60, 90, 120 kg N ha<sup>-1</sup>) and three P rates (0, 60, 120 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) arranged in a randomized block design with four replications. Each plot was 4×5 m, and was flooded throughout the experiment. Occasional spraying against pests and diseases was needed. Three-week-old seedlings were transplanted by hand with a 0.15×0.15 m spacing. The P and K fertilizers were Longthanh superphosphate (16% P<sub>2</sub>O<sub>5</sub>) and KCl

(50% K<sub>2</sub>O at 60 kg K<sub>2</sub>O ha<sup>-1</sup>). Nitrogen was applied as urea (46.8% N) in three splits. The first N split and all P and K were applied at transplanting. The second and third N splits were at 10 and 20 days after transplanting. The <sup>15</sup>N labelled fertilizer was urea, at 1.5% atom <sup>15</sup>N excess. Each <sup>15</sup>N microplot was 0.8×0.8 m, surrounded by a 0.35-m deep galvanized iron sheet inserted into the soil to a depth of about 0.2 m with 0.15 m above the soil surface, effectively preventing runoff. Each microplot contained twenty-five plants.

A single harvest was made, at maturity, by cutting the shoots at 3 to 5 cm above the ground. Yield (harvest area: 12 m<sup>2</sup> per plot) and biomass were recorded. The <sup>15</sup>N samples were collected from the microplots (nine hills each). The straw and panicles were separated. After chopping, sub-samples were taken and dried in an oven at 70°C for 48 h and milled for analysis. Total P was determined using the phosphovanado molybdate yellow method [3]. Plant samples were analysed for %N by Kjeldahl digestion [4]. The <sup>15</sup>N enrichment (NOI-6e Emission Spectrometer) was used to determine N uptake and use of the various fertilizer treatments [5]. Total P accumulated in the plants was calculated. The percent of fertilizer-N utilization (%FNU), or recovery, was calculated as:

$$\%FNU = \frac{(\text{Fertilizer N uptake})}{(\text{Fertilizer N applied})} \times 100$$

An RCBD two-factor factorial was used to statistically assess the effects of N and P rates on biomass and yield production, total P uptake and N uptake, and P and N utilization. The interactive effects of N×P were also determined. When a significant ( $P < 0.05$ ) treatment effect was found, least significant difference (LSD) values were calculated in order to compare treatment means.

### 3. RESULTS AND DISCUSSION

#### 3.1. Dry-matter yields and yield components

The response of rice grain and dry matter to the application of the four N and three P rates are presented in Tables I and II. Nitrogen and P fertilization increased total dry matter yields over the no-fertilizer control. For N fertilization (Table I) the highest grain-yield was obtained with the first rate of 60 kg N ha<sup>-1</sup>, and it decreased thereafter, with 90 and 120 kg N ha<sup>-1</sup>. Straw yield increased with N rates up to 120 kg N ha<sup>-1</sup>, although the yield over that obtained with 90 kg ha<sup>-1</sup> was not significant. For the P fertilization, straw and grain yield increases were obtained up to the highest rate of 120 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (Table II). There was no significant N×P interaction.

TABLE I. EFFECT OF RATE OF APPLICATION OF N ON DRY-MATTER YIELDS OF STRAW AND GRAIN OF cv. IR66

N rate (kg N ha <sup>-1</sup> )	Dry matter yield (ton ha <sup>-1</sup> )			
	Straw	Increase over control (%)	Grain	Increase over control (%)
0	2,389	—	3,256	—
60	2,439	2.1	4,347	33
90	2,729	14	4,003	23
120	2,779	16	3,495	7.2
LSD <sub>0.05</sub>	0.283		0.291	
CV%	12.7		8.75	



TABLE II. EFFECT OF P RATE ON DRY MATTER YIELDS OF STRAW AND GRAIN AND PANICLE NUMBER OF cv. IR66

P rate (kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )	Dry matter yield (ton ha <sup>-1</sup> )				No. of Panicles m <sup>-2</sup>
	Straw	Increase over control (%)	Grain	Increase over control (%)	
0	2,476	—	3,606	—	363
60	2,559	3.4	3,579	0.0	378
120	2,912	17.6	3,941	9.3	398
LSD <sub>0.05</sub>	283		0.291		19.0
CV%	12.7		8.75		6.10

In other studies, P deficiency affected grain yield by reducing tillering [6,7]. The number of panicles was increased at flowering (75 day after transplanting) (Table II) by application of P. The highest panicle number was observed at 120 kg N ha<sup>-1</sup>, which also produced the highest grain yield. Grain-yield reduction at low P supply was mainly due to inhibition of tillering, leading to fewer panicles per plant. This finding is in agreement with results of Romer and Schilling [8] and Horst and Wiesler [6] that grain yield is more affected by P nutrition before than after anthesis.

### 3.2. Total P uptake by rice

There were significant differences in P accumulation between N levels (Table III); total P was highest at 90 kgN ha<sup>-1</sup> and decreased at 120 kg N ha<sup>-1</sup>, a trend similar to that of grain yield. This indicates that the P uptake was highly correlated with grain yield and depends on N-fertilizer levels.

Phosphorus uptake was increased significantly with increasing of P supply (Table IV). The highest grain P yield was again observed at 120 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. At high P supply, the higher P content of the grains can be explained mainly by more-efficient P uptake [6]. The P concentration of the straw most sensitively responded to the P supply (Table IV). These results support conclusions by Horst and Wiesler [6] that the source capacity of plants is greatly reduced under P deficiency.

TABLE III. EFFECT OF N SUPPLY ON P YIELD OF cv. IR66 AT MATURITY STAGE

N rate (kg N ha <sup>-1</sup> )	Total P yield (kg P ha <sup>-1</sup> )		
	Grain	Straw	Total
0	10.5	4.94	15.2
60	14.7	4.16	18.9
90	14.7	4.80	19.5
120	11.2	5.71	16.7
LSD <sub>0.05</sub>	1.93	0.74	2.09
CV%	18.1	18.4	14.3

TABLE IV. EFFECT OF P SUPPLY ON  
P YIELD OF cv. IR66 AT MATURITY

P rate (kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )	Total P yield (kg P ha <sup>-1</sup> )		
	Grain	Straw	Total
0	12.2	4.46	16.7
60	12.5	4.61	17.1
120	13.8	5.63	19.4
LSD <sub>0.05</sub>	NS	0.64	1.81
CV%	18.1	18.4	14.3

### 3.3. N uptake and N-use efficiency

The influence of applied N and P on N uptake and N-use efficiency by rice cv. IR66 is shown in Tables V and VI, respectively. Straw and grain total N increased with the rate of fertilizer N. Significant increases in %Ndff values of straw and grain were observed. Fertilizer-N recovery by the rice ranged from 26 to 32%, the highest value being for the 90 kg N ha<sup>-1</sup> rate. These values are consistent with those summarized by Craswell and Vlek [9] in which recoveries of <sup>15</sup>N ranged from 7% to 68%. Response of rice to N is affected by varietal characteristics, soil conditions, weather, and management practices [10]. To maximize N-use efficiency, the appropriate application of N fertilizer should be determined. The %Ndff values were, in general, similar in grain and straw. However, the recovery in straw was lower than in the grain. Applied P increased the straw and grain total N, but not the %Ndff. Fertilizer-N recovery by the rice plants was lowest (27%) for the control without P fertilizer application, and increased with fertilization up to 32% with 120 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. This shows that P nutrition plays an important role in influencing N-use efficiency of rice grown in an acid-sulphate soil.

TABLE V. EFFECT OF N RATE ON TOTAL N, %Ndff, AND %N RECOVERY BY cv. IR66

N rate (kg N ha <sup>-1</sup> )	Total N uptake (kg N ha <sup>-1</sup> )	%Ndff		Fertilizer N yield (kg N ha <sup>-1</sup> )	Fertilizer N utilization (%)
		Straw	Grain		
60	71.7	26.6	28.0	18.6	30.5
90	87.5	33.1	35.9	30.3	32.2
120	81.7	37.7	40.7	31.8	26.1
LSD <sub>0.05</sub>	2.53	1.91	1.98	0.143	1.40
CV%	10.1	7.0	6.73	6.27	5.77

TABLE VI. EFFECT OF P RATES ON TOTAL N, %Ndff AND %N RECOVERY BY cv. IR66

P rate (kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )	Total N uptake (kg N ha <sup>-1</sup> )	%Ndff		Fertilizer N yield (kg N ha <sup>-1</sup> )	Fertilizer N utilization (%)
		Straw	Grain		
60	73.7	31.7	33.7	24.4	30.5
90	78.5	32.5	35.1	26.2	32.2
120	88.3	33.2	35.8	30.2	26.1
LSD 5%	2.53	NS	NS	0.143	1.4
CV%	10.1	7.00	6.73	6.27	5.77

#### 4. CONCLUSIONS

Application of N and P fertilizers increased N and P uptake and grain yield over the control in all treatments. The maximum yield and highest fertilizer N utilization were attained with the combination of 90 kg N ha<sup>-1</sup> and 120 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. The N recovery was influenced by the amount of P fertilizer applied. Increased levels of P enhanced the N-utilization efficiency, which was highest at 120 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. These results form a basis for further investigation to improve the N recovery in rice-cropping system and to gather data for socio-economic analyses of N and P fertilization of rice in this area.

#### ACKNOWLEDGEMENTS

The authors thank the IAEA for supplying the <sup>15</sup>N analyser and <sup>15</sup>N labelled fertilizers under TC project VIE 5/011. This study was supported and funded by the Viet Nam Atomic Energy Commission. We are grateful to Dr. F. Zapata for improving the manuscript.

#### REFERENCES

- [1] VU, C.T., The Map Zones of Effect of Nitrogen and Phosphorus for Rice Growing on the Mekong Delta (1995).
- [2] NGUYEN, D.N., The efficiency and the fixing ability of phosphorus in acid sulfate soils of Dong Thap Muoi, Vietnam Soil Sci. **5** (1995) 61–68.
- [3] FOGG, D.N., WILKINSON, N.T., The colourimetric determination of phosphorus, Analyst (London) **83** (1958) 406–416.
- [4] EASTIN, F.F., Total nitrogen determination for plant materials, Anal. Biochem. **85** (1978) 591–594.
- [5] ZAPATA, F., Isotope Techniques in Soil Fertility and Plant Nutrition Studies of Soil-Plant Relationships, IAEA, Vienna (1990).
- [6] HORST, W.J., WIESLER, F., “Genotypic differences in acquisition and utilization of phosphorus in wheat”, Isotope Studies on Plant Productivity, IAEA-TECDOC-889, Vienna (1996) 73–88.
- [7] ZAHARAH, A.R., et al., “Evaluation of three phosphate fertilizer sources for sweet corn production”, Nuclear Techniques in Soil-Plant Studies for Sustainable Agriculture and Environmental Preservation, IAEA, Vienna (1995) 145–156.
- [8] ROMER, W., SCHILLING, G. Phosphorus requirements of the wheat plant in various stages of its life cycle, Plant Soil **91** (1986) 221–229.
- [9] CRASWELL, E.T., VLEK, P.L.G., “Fate of fertilizer nitrogen applied to wetland soil”, Nitrogen and Rice, IRRI, Los Baños (1979) 175–192.
- [10] DE DATTA, S.K., MAGNAYE C.P., Survey of forms and sources of fertilizer nitrogen for flooded rice, Soil Fert. Abstr. **32** (1969) 103–109.

# **SOIL ORGANIC MATTER DYNAMICS AND NUTRIENT CYCLING**

(Session 2)



**Keynote Address****C AND N CYCLING IN SOIL: ADVANCES IN THE APPLICATION OF  $^{13}\text{C}$  AND  $^{15}\text{N}$  TECHNIQUES**

S. RECOUS, B. MARY  
 Institut National de la Recherche Agronomique (INRA),  
 Unité d'Agronomie, Laon,

B. NICOLARDOT  
 INRA,  
 Unité d'Agronomie,  
 Reims

France

**Abstract**

Isotopes such as  $^{13}\text{C}$ ,  $^{14}\text{C}$ , and  $^{15}\text{N}$  are uniquely useful tools for investigating the cycling of soil organic matter and to trace the fate of nutrients in agroecosystems. In this paper, we examine how C and N interact during decomposition of organic matter, and how these interactions are fundamental to the understanding of C and N cycling, even considered separately. Also, we examine and compare various methods involving  $^{15}\text{N}$  tracing to quantify net and gross N fluxes associated with the decomposition of added and soil organic matter. Recent research efforts to understand the relation between soil organic matter and soil structure, and associated  $^{13}\text{C}$ -tracing techniques, are discussed.

**1. INTRODUCTION**

Soil organic matter (SOM) is essential in maintaining soil fertility. Nutrient release for uptake by crops is particularly crucial in farming systems for which nutrient input is mainly or solely based on the return of crop residues [1]. In countries where intensive agriculture is based on high inorganic nutrient inputs, the systems of management of soils, crops, and nutrients are changing due to requirements to protect the environment (e.g. development of cover crops during fallow periods to prevent leaching), increases in recycling of organic wastes onto soils, and the reduction of soil cultivation for economic reasons [2]. Nutrient management in intensive cropping systems is increasingly dependent on added organic matter, and sources of organic residues returned to soil, and conditions necessary for decomposition (quality, location, date of incorporation, etc.) are becoming more variable.

Soil organic matter represents a major pool of C within the biosphere, and may act as both a source of, and a sink for, C during global environmental change. Changes in climate, in land use, and in management are likely to influence the rates of accumulation and decomposition of C in SOM [3]. Consequently, studies on SOM decomposition and on the associated C and N cycles remain necessary, particularly regarding the effects of biochemical quality of residues, and method of placement in soil.

Methods developed to study SOM cycling vary with the objectives. Some research aims at describing the C and N cycling in order to understand, in the short term (days to months), the dynamics of decomposition of organic matter, the release of nutrients, to examine synchrony with crop demand, and to study and prevent losses (gaseous emissions, leaching). Other research has focused on net inputs of organic matter and medium to longterm balances (months to years), in order to modify some physical, biological and/or chemical properties of soils. Most of the research has dealt either with N (availability, losses) or with C (e.g. soil-structure relationships), while some work has analysed jointly the dynamics of C and N, particularly for modelling purposes. Consequently, a range of concepts, methods, and models have been developed and co-exist in the field of C and N cycling studies.

## 2. CARBON AND N INTERACTIONS DURING THE DECOMPOSITION OF ORGANIC MATTER

During the decomposition of organic matter, simultaneous assimilation of C and N by the heterotrophic microflora causes the mineralization-immobilization turnover of N in soil to be linked to the cycling of C. The C-assimilation rate depends on the rate of decomposition of plant material and the assimilation yield of the decomposed C by the microflora. The C flow and the C:N ratio of the decomposers then determine the N assimilation requirements. The decomposition of organic matter (fresh or humified) leads either to the net release of inorganic N in soil, or to temporary net immobilization. The dynamics as well as the net amounts of N immobilized vary greatly according to the nature of plant residues (chemical characteristics) and Z content of the various organic pools. For example Mary et al. [4] reported a net mineralization of +15 mg N g<sup>-1</sup> added C for wheat leaves, a net immobilization of -28 mg N g<sup>-1</sup> added C for wheat straw, and -72 for root mucilage. The calculations obtained from different experiments indicate a rather narrow range of values for N immobilization for a given substrate, from 26 to 31 mg N g<sup>-1</sup> added C for wheat straw decomposing under non-limiting conditions of N availability [4].

There is general agreement that the threshold between net release of N and net immobilization of mineral N in soil occurs with a residue C:N ratio of about 25 [5,6], above which the microbial N demand during decomposition is no longer fulfilled by the N in the crop residue. Indeed, dynamics of net accumulation of N varies with time and consequently the net effect of incorporating a C source on soil N depends mostly on the time scale during which the N effect is considered. In the short term it appears that only a few residues lead to the rapid net release of N; most provoke a phase of net immobilization, followed by a phase of net mineralization, the magnitude and the duration of which depend of the overall N content of soil plus residue and the biochemical quality of the residue itself [6].

Conversely, the availability of N in soil also controls the decomposition and dynamics of C, both in the short and in the long term [7]. This fact is long established for various types of plant residue. In the short term, little N availability restricts the growth of heterotrophs. This result is not surprising since the C:N ratio of soil micro-organisms is less than those of most plant residues and, therefore, the external source of N can be rapidly exhausted. In the longer term, N availability also influences the decay process and may exert a negative effect on decomposition, because high N availability is known to inhibit the synthesis of ligninolytic enzyme systems [8]. The complex interactions between N and type of residue, type of decomposer, and the chemical reactions during humification have been described [7].

For plant residues of high C:N ratio, such as straw, it has been demonstrated that the shortage of N not only reduces initial rates of decomposition of organic matter but also modifies the relationships between decomposed C and immobilized N [9–11]. Several hypotheses exist to explain these changes, such as those in microbial succession, the adaptation of internal N content of fungi, the modification of energy allocated to growth or to maintenance, and increases in the rate of biomass recycling. These have been arrived at not only using controlled conditions, but also in the field [4]. When N was abundant, a ratio of 32 mg N immobilized g<sup>-1</sup> added straw-C was calculated, and this value was similar to the immobilization ratio obtained in conditions optimal for decomposition [9]. When N was limiting, the immobilization ratio decreased accordingly, to 24 and 13 mg N g<sup>-1</sup> added C. The authors concluded that, in field conditions, the dynamics of organic matter is very often driven by the availability of N at the site of decomposition. This is the case in situations of low soil-N content at the time of residue-incorporation and/or of uneven spatial distribution of crop residues in the soil. Trinsoutrot et al. [12] and Dejoux et al. [13] compared the dynamics of N accumulation in soil during decomposition of rape residues, both under laboratory and field conditions. They showed that incubation in “optimal” conditions underestimated significantly the net availability of N compared to field decomposition. They assumed that this was due to lower immobilization in field conditions related to availability of N and/or reduced residue-soil contact.

The residue-N content forms part of the overall N availability, and often controls the dynamics of decomposition. Consequently the effect of residue-N content cannot be analysed independently of the overall N availability (soil N + residue N), if total N availability is limiting. When N availability is limiting of the microbial N demand, N availability controls decomposition and the observed kinetics of mineralization of residue-C do not allow the effect of biochemical quality to be assessed or distinguished from the effects of N availability. Trinsoutrot et al. [6] emphasized the need to assess separately the effect of biochemical composition of the organic residues and the effect of N content. Simultaneous and mutual control of C and N dynamics is, therefore, complex. This suggests that parameters associated with C and N cycling, obtained under optimal conditions of incubation, might fail to accurately predict N dynamics in field conditions due to the effect of N availability. With models parameterized in controlled conditions, it should, therefore, be considered whether the parameters were obtained in limiting or non-limiting conditions of N availability, and necessarily should include a function that affects not only potential decay rates but also C-N relationships [14].

### 3. QUANTIFICATION OF N BIOTRANSFORMATIONS

Much work has been dedicated to assessing the effects of biochemical quality of added organic matter to soils, to predict the dynamics of nutrient release, or in the longer term to determine the net input to SOM. Indeed this is difficult owing to the complex interactions between the various sources of N involved during the decomposition of the added C. During decomposition, the sources of N for the microbial biomass can be the residue itself, the mineral N already present in soil or recently mineralized, and the recycling biomass. It is often assumed that N coming from the residue and from the recycling biomass is mineralized before being assimilated by the newly formed biomass. However it has been shown that a significant amount of organic or mineral N can be assimilated by the decomposers either developing on the particulate matter itself or in the soil, without first entering the soil mineral N pool.

Measuring the variations in soil inorganic N with time allows the quantification of net immobilization and net mineralization. This approach, termed “N balance,” is based on the difference in cumulative N mineralization between a soil amended with a C substrate and an unamended soil (or control), over time. If the difference in net accumulation is expressed as a function of the added N, then it is assumed that the mineralization of the native SOM is unaffected by the addition of organic matter into the soil, which is debatable. Nevertheless such an N balance takes into account all interactions, i.e. it is an unbiased picture of the net effect of organic substrate addition on the dynamics of N in soil. However N release in practical situations is often rather small compared with total crop N uptake so the precision of measurement can be poor when crop N uptake is a term of the balance.

Owing to the complexity of the various N fluxes, often acting in opposite directions (release or consumption of N), <sup>15</sup>N tracing has been central to understanding the mechanisms involved, and it is still the sole option to quantify gross N biotransformations. Several methods involving <sup>15</sup>N have been developed. The widely used approach consists of adding homogeneously <sup>15</sup>N-labelled residues. Labelling the added organic source allows the fate of the added N to be traced in the various soil pools and the recovery of added <sup>15</sup>N by plants to be quantified. For example, Ladd et al. [15], Rees et al. [16], and Thomsen and Christensen [17] observed a rather low availability of added N to a subsequent crop (7–26% of the added <sup>15</sup>N), indicative of immobilization and stabilization in the soil. Alternatively, or in parallel, the soil inorganic N pool can be labelled with <sup>15</sup>N. This allows the quantification over time of the net immobilization of soil N during the decomposition process. This approach shows that soil N contributes much to the microbial immobilization process, to an extent that can be closely related to the residue-N content itself [6]. In such experiments, the observed increase in the <sup>15</sup>N enrichment of residues—initially unlabelled—during decomposition, demonstrated that the decomposers are growing on the particulate matter itself while their N requirements are fulfilled from the surrounding labelled soil N [18,19]. Lastly, Watkins and Barraclough [20], and others, have proposed a method of calculation based on “paired” labelled treatments (either the residue is labelled or the soil)—also called “cross labelling design”—and proposed equations that estimate the direct assimilation of residue-N by the soil microflora.



The information provided by the various approaches is rather different, as shown, for example, for rape residues [12]. Only a small proportion (14–27%) of the  $^{15}\text{N}$  derived from the residues was accumulated in soil after 168 days, whereas most of the added C (80%) was decomposed. Most of the residue-N released during decomposition was assimilated by the decomposing microorganisms or remained in a more recalcitrant soil organic fraction. However, net mineralization of the added  $^{15}\text{N}$  was higher than net mineralization calculated from the N balance between amended and control soil, showing that soil N was immobilized in the amended treatment instead of the residue-N, i.e. that substitution effects occurred between labelled and unlabelled sources as immobilization was enhanced by C addition [21]. Consequently, assessing the mineralization of residue-N through the accumulation of  $^{15}\text{N}$  in soil (or accumulated in soil + taken up by plants) both underestimates the actual decomposition of the residue-N and overestimates the actual availability of that mineralized N. Indeed substitution effects interfere much when interpreting N fluxes with the use of  $^{15}\text{N}$ , as pointed out earlier [22]. Hood et al. [23] proposed an alternative method to minimize substitution effects. The method consists of pre-labelling of the SOM by immobilizing some inorganic  $^{15}\text{N}$  added together with a C substrate (e.g. cellulose) a considerable time before adding unlabelled residues. It is hypothesized that by pre-labelling the soil with  $^{15}\text{N}$ , the inorganic N pool and the incoming N from basal mineralization are of a similar  $^{15}\text{N}$  enrichment and are not altered by N immobilization due to residue addition, thus overcoming the problems associated with pool substitution. Then mineralization of unlabelled residue N is estimated from the extent of dilution of the mineral N pool revealed by the plant N pool. This new method requires further testing and adaptation to field use.

Research dealing with the quantification of “gross N fluxes” received much attention during the last decade. Indeed, net mineralization is the outcome of two oppositely directed soil processes, ammonification (i.e. gross mineralization) and microbial assimilation (i.e. gross immobilization) that fills or depletes the ammonium pool. Together with nitrification, these three processes form the so-called mineralization-immobilization turnover (MIT) in soil [24]. Knowledge of the actual rates of these processes can be very useful in understanding how soil and crop management affects N turnover, or to evaluate some of the concepts introduced in C-N biotransformation models, as most of them first describe gross N transformations, from which net mineralization is calculated. Only  $^{15}\text{N}$ -isotope tracing allows the two processes to be calculated separately. Based on the “dilution principle,” gross N mineralization is calculated—after homogeneously labelling the soil  $\text{NH}_4^+$  pool with  $^{15}\text{N}$ —from the decrease in  $^{15}\text{N}$  enrichment and the change in the size of the soil ammonium pool as microorganisms mineralize native SOM- $^{14}\text{N}$  to  $^{14}\text{NH}_4^+$  [25]. Nitrification and immobilization can be calculated from enrichment in  $^{15}\text{N}$  of the nitrate or organic N pool, or from the consumption of the ammonium. Analytical equations and numerical models have been proposed to calculate the N fluxes [26,27].

The gross-flux method differs fundamentally from the previous ones, because it aims at tracing the whole soil mineral pool and not only the fate of the added label N. Therefore, the method involves conditions (homogeneity of labelling, equilibrium between added and native N, etc.) that are difficult to obtain, and sometimes contradictory. Consequently, much research has already looked at the best method to apply the label (e.g. spray vs. injection) to obtain homogeneity in the distribution of the label in soil cores, and to determine optimal duration for incubation [28,29]. Despite these difficulties, the approach has yielded useful information on estimates of actual gross fluxes under grasslands [30,31], forests [32,33], the stratification of gross mineralization in soil [34], the effect of crop history on gross mineralization [35], and changes in gross mineralization and immobilization during decomposition of crop residues [36]. An alternative use of the gross-flux technique was developed more recently to study the effect of factors on the N processes. In this latter case, the gross-flux technique is used in controlled conditions to assess the effect of various factors on potential gross mineralization, immobilization, or nitrification (e.g. effect of soil disturbance, soil re-moistening, soil temperature) [37]. The gross-flux approach gives information on instantaneous rates of transformations. In that respect, it is a useful tool for assessing relationships between processes (e.g. C and N mineralization) [36].

In conclusion, a very different picture of the N biotransformations is obtained when comparing N balances, net  $^{15}\text{N}$  release, gross N fluxes, or N fluxes calculated with the use of a model [12]. This reveals the different abilities of the methods to shed light on various parts of the underlying processes

involved in soil-N cycling. Consequently, the interpretation of the processes, particularly with the use of  $^{15}\text{N}$ , requires much care, despite the apparent simplicity.

#### 4. CARBON DYNAMICS AND RELATIONS WITH SOIL STRUCTURE

Soil structure appears to be one of the dominant factors controlling the decomposition of SOM [38]. Tillage breaks soil aggregates and thereby exposes SOM that was previously protected within the aggregate structure. On the other hand, organic matter is a major agent that stabilizes aggregates, and decreases in SOM content on cultivation are thought to be responsible for the deterioration of soil structure. Therefore, one of the main challenges was to unravel the relationships between soil structure and organic matter and to clarify the concept of physical protection. It has involved examining the complex relationships between distribution of SOM relative to soil architecture, the spatial location of the microorganisms and of the C substrates in soil and their interaction with the soil matrix. Several approaches based on the investigation of C and  $^{13}\text{C}$  turnover (either with the enriched or natural abundance techniques) have been developed.

Studies on C dynamics examine either the short term fate of C substrates added to soil or long term effects, and the required experimental approaches are rather different. The techniques used in the past with radioisotopic  $^{14}\text{C}$  have increasingly being applied to  $^{13}\text{C}$  studies to trace the added C in various pools, using labelled sources such as crop residues or simple macromolecules (glucose, cellulose), and this has been made possible by the improvement of analytical techniques. The information provided by the use of  $^{13}\text{C}$  is similar to that obtained with  $^{14}\text{C}$  tracing [e.g. 39], but it is safe for use and this is crucial for developing experiments in natural conditions. It also makes the combined analyses of C, N,  $^{13}\text{C}$ , and  $^{15}\text{N}$  possible. The higher sensitivity of the enriched label technique, compared to the natural-abundance approach, allows the study of short term evolution of the added C in soil, for example measuring rates of mineralization, the distribution of the residual straw in the soil fractions, and establishing relationships between instantaneous C and N processes [40,41].

Carbon evolution can also be measured precisely in long term experiments, and the method of isotopic tracing of natural  $^{13}\text{C}$  based on the difference in  $^{13}\text{C}/^{12}\text{C}$  ratios between  $\text{C}_3$  and  $\text{C}_4$  plants has proved to be particularly suitable for identifying the origin of the C. Carbon derived from a  $\text{C}_4$  plant can be distinguished from that of  $\text{C}_3$  plant by its isotopic composition. For example, the cropping of maize (a  $\text{C}_4$  plant) on soils that had previously supported only  $\text{C}_3$  plants (mainly trees and temperate vegetation) provides a natural label of SOM. This label can be used to distinguish young SOM derived from the recent crop and older SOM from the previous  $\text{C}_3$  crops [42]. This method has been used successfully to quantify the contributions of various crops to the SOM dynamics, i.e. the shift from forest or native vegetation to cultivated crops. Cultivation of a virgin soil has been reported extensively to cause a drop in SOM concentration. Most studies have shown that decline of C concentration is very rapid in the first years following forest clearing or grassland conversion [43]. The kinetic studies based on natural tracing with  $^{13}\text{C}$  thus introduced the concept of SOM pools that are de-protected by cultivation.

First,  $^{13}\text{C}$  natural tracing techniques based on change in land use, have been combined with soil physical fractionation procedures (soil aggregates or soil particles) using treatments that disrupt the soil structure to different degrees and thereby select units of various physical stability [44]. Soil organic matter fractions may also be physically separated on the basis of degree of association with minerals using differences in density. Based on the natural  $^{13}\text{C}$  analyses of the various C fractions, functional pools of soil organic matter have been proposed based on the calculation of their turnover time. The general picture is one of a much slower turnover rate in the clay and/or silt size separates, ascribed to protective mechanisms exerted by the larger and active surfaces of the minerals.

The aggregate hierarchy, based on the conceptual model of Tisdall and Oades [45] has been used as a framework to investigate the correlation between a reduction in aggregation and loss of SOM with cultivation [46,47]. The general approach involves the physical separation of stable macro-aggregates ( $>250\text{ }\mu\text{m}$ ) (slaking-resistant macro-aggregates, obtained from air-dried and fast-rewetted soils) and micro-aggregates ( $53\text{--}250\text{ }\mu\text{m}$ ) separated by wet-sieving. This approach, combined with the isotopic  $^{13}\text{C}$  signature of the associated C, has been developed mainly in situations contrasting in terms of land

use (native vegetation vs. no-tilled system vs. conventional tillage) [e.g. 48–50], or the amount and characteristics of added N or C [e.g. 51,52]. In temperate soils, it has been demonstrated that more organic matter binds micro-aggregates (53–250  $\mu\text{m}$ ) into macro-aggregates ( $>250 \mu\text{m}$ ), which, as a consequence, have a larger C content. For example, Puget et al. [53] and Six et al. [54] showed that slaking-resistant macro-aggregates were enriched in C compared to dry-sieved macro-aggregates or to micro-aggregates, and that the C content increased with the size of aggregates. Six et al. [55] examined the distribution of C and  $^{13}\text{C}$  with increasing disruptive energy applied to soil, and related the C lost from increasing cultivation intensity with the higher proportions of stable macro-aggregates. These results supported the conceptual model proposed earlier [45], and demonstrated that fragments of plant debris account for the formation and stabilization of macro-aggregates via the production of mucilaginous materials and fungal hyphae during microbial growth [56,57]. The proportions of micro- and macro-aggregates, the size of macro-aggregates and their turnover rates all vary with the amount, the chemical nature, size, geometry and mode of deposition of the particulate organic matter [58]. Aggregation then influences the concentration and turnover of the new soil organic matter by limiting the accessibility of C substrates to microbes and fauna. The delta  $^{13}\text{C}$  analysis suggested that the larger C contents in stable macro-aggregates were due to young  $\text{C}_4$ -derived organic C, and it was concluded that young organic matter was responsible for macro-aggregate stability [53]. Six et al. [55] hypothesised that microbial-derived SOM should be closely linked to that of aggregates due to the role of microbial products in binding soil into aggregates and protecting them. They attempted to isolate a pool of organic matter derived from microbes with an intermediate turnover, that is lost upon cultivation, also called the Enriched Labile Fraction [55]. However it appears that the increasing C concentrations with larger aggregates may depend on mineralogy and climate [1,55].

This methodology has been successful in identifying fractions of SOM, such as particulate organic matter and light fractions derived from plants, that are more sensitive to cultivation and management practices than total organic C or N [58]. It is also essential for the understanding of consequences for nutrient dynamics, in the shorter term, as it has been demonstrated that the gross N biotransformations can be rather closely related to the dynamics of the particulate organic matter in soils [36,59].

To investigate the relationship between the location of microorganisms and the organic matter within the soil structure, another approach consisted of quantifying the soil biomass in the fractions or aggregates separated physically [60]. Chotte et al. [61], using labelled material, found the sites of microbial assimilation of soluble substrates to be micro-aggregates of between 2 and 50  $\mu\text{m}$ , and the sites of microbial assimilation of particulate substrates to be particulate residues and micro-aggregates of 2 to 50  $\mu\text{m}$  diameter. Gaillard et al. [19] adopted a different approach to analyse the gradients of C, N and microbial activity in the detritusphere. Based on the spatial distribution of residue-derived  $^{13}\text{C}$  and  $^{15}\text{N}$ , they showed the strong spatial heterogeneity at the millimetre scale, and identified the sites of microbial assimilation.

## 5. CONCLUSIONS

Major advances on the dynamics of SOM have been made, particularly in terms of the interactions between C and N cycling, interactions between quality and location of organic matter added to soil, and interactions between organic matter turnover and soil structure. The development of isotope techniques, in combination with modelling tools, appears very powerful in understanding such interactions, for testing new concepts, and for predicting the evolution of C and N in soils [e.g. 62,63]. However, the scarcity of measurements of below ground C and N inputs, either from root turnover or from rhizodeposition has been identified as one of the major reasons for lack of predictions of the dynamics of C and N both in the short and in the long term in various situations [64]. Several investigations have suggested that root inputs to soil-plant systems are much larger than what can be estimated from soil samplings and root extractions. The decomposition of below ground organic inputs also differs radically from the decomposition of above ground parts as a result of their chemical and physical characteristics and location. Therefore, recent methods [e.g. 65,66] that aim at more precisely quantifying the N or C inputs from roots, their effects on soil microflora, their contributions to soil respiration or their subsequent evolution in soil are promising, and will probably receive much attention. This appears as a major challenge for future research.

## REFERENCES

- [1] MERCKX, R., et al., "The role of soil organic matter in maintaining soil fertility", Sustaining Soil Fertility in West-Africa, ASA, Special Publication, Madison (in press).
- [2] JENKINSON, D.S., The impact of humans on the nitrogen cycle, with focus on temperate arable agriculture, Plant Soil, Special Issue of the 11<sup>th</sup> N Workshop (in press).
- [3] LAL, R., et al., "Pedospheric processes and the carbon cycle", Soil Processes and the Carbon Cycle (LAL, R., et al., Eds.), CRC Press, Boca Raton (1998) 1–8.
- [4] MARY, B., et al., Interactions between decomposition of plant residues and nitrogen cycling in soil, Plant Soil **181** (1996) 71–82.
- [5] PAUL, E.A., CLARK, F.E., Soil Microbiology and Biochemistry, Academic Press, San Diego (1989).
- [6] TRINSOUTROT, I., et al., Biochemical quality of crop residues and carbon and nitrogen mineralization kinetics under nonlimiting nitrogen conditions, Soil Sci. Soc. Am. J. **64** (2000) 918–926.
- [7] FOG, K., The effect of added nitrogen on the rate of decomposition of organic matter, Biol. Rev. **63** (1988) 433–462.
- [8] KEYSER, P., et al., Ligninolytic enzyme system of *Phanerochaete chrysosporium*: synthesized in the absence of lignin in response to nitrogen starvation, J. Bacteriol. **135** (1978) 790–797.
- [9] RECOUS, S., et al., Soil inorganic N availability: effect on maize residue decomposition, Soil Biol. Biochem. **27** (1995) 1529–1538.
- [10] GREEN, C.J., BLACKMER, A.M., Residue decomposition effects on nitrogen availability to corn following corn or soybean, Soil Sci. Soc. Am. J. **59** (1995) 1065–1070.
- [11] HENRIKSEN, T.M., BRELAND, T.A., Nitrogen availability effects on carbon mineralization, fungal and bacterial growth, and enzymes activities during the decomposition of wheat straw in soil, Soil Biol. Biochem. **31** (1999) 1121–1134.
- [12] TRINSOUTROT, I., et al., C and N cycling during the decomposition of <sup>13</sup>C<sup>15</sup>N labeled oilseed rape residues: effect of their biochemical characteristics and N content, Soil Biol. Biochem. **32** (2000) 1717–1730.
- [13] DEJOUX, J.F., et al., Fate of nitrogen from winter-frozen rapeseed leaves: mineralization, fluxes to the environment and uptake by rapeseed crop in spring, Plant Soil **218** (2000) 257–272.
- [14] HADAS, A., et al., Reduced CO<sub>2</sub> release from decomposing wheat straw under N-limiting conditions: simulation of carbon turnover, Eur. J. Soil Sci. **49** (1998) 487–494.
- [15] LADD, J.N., et al., Distribution and recovery of nitrogen from legume residues decomposing in soils sown to wheat in the field, Soil Biol. Biochem. **13** (1983) 251–256.
- [16] REES, R.M., et al., The release and plant uptake of nitrogen from some plant and animal manures, Biol. Fert. Soils **15** (1993) 185–193.
- [17] THOMSEN, I.K., CHRISTENSEN, B.T., Availability to subsequent crops and leaching of nitrogen in <sup>15</sup>N-labelled sugarbeet tops and oilseed rape residues, J. Agric. Sci. (Camb.) **126** (1996) 191–199.
- [18] CHESCHIRE, M.V., et al., The immobilization of nitrogen by straw decomposing in soil, Eur. J. Soil Sci. **50** (1999) 329–341.
- [19] GAILLARD, V., et al., C, N and microbial gradients induced by plant residues decomposing in soil, Eur. J. Soil Sci. **50** (1999) 567–578.
- [20] WATKINS, N., BARRACLOUGH, D., Gross rate of N mineralization associated with the decomposition of plant residues, Soil Biol. Biochem. **28** (1996) 169–175.
- [21] HOOD, R.C., et al., A comparison of direct and indirect <sup>15</sup>N isotope techniques for estimating crop N uptake from organic residues, Plant Soil **208** (1999) 259–270.
- [22] HART, P.B.S., et al., Influence of pool substitution on the interpretation of fertilizer experiments with <sup>15</sup>N, Soil Sci. **37** (1986) 389–403.
- [23] HOOD, R.C., et al., Estimating crop N uptake from organic residues using a new approach to the <sup>15</sup>N isotope dilution technique, Plant Soil **223** (2000) 33–44.
- [24] JANSSON, S.L., PERSSON, J., "Mineralization and immobilization of soil nitrogen", Nitrogen in Agricultural Soils (STEVENSON, F.J., Ed.), ASA, Madison (1982) 229–252.

- [25] KIRKHAM, D., BARTHOLOMEW, W.V. Equations for following nutrient transformations in soil utilising tracer data, *Soil Sci. Soc. Am. J.* **18** (1954) 33–34.
- [26] BARRACLOUGH, D. The use of mean pool abundances to interpret  $^{15}\text{N}$  tracer experiments. I. Theory, *Plant Soil* **131** (1991) 89–96.
- [27] MARY, B., et al., A model for calculating nitrogen fluxes in soil using  $^{15}\text{N}$  tracing, *Soil Biol. Biochem.* **30** (1998) 1963–1979.
- [28] DAVIDSON, E.A., et al., Measuring gross nitrogen mineralization, immobilization, and nitrification by  $^{15}\text{N}$  isotopic pool dilution in intact soil cores, *J. Soil Sci.* **42** (1991) 335–349.
- [29] MURPHY, D.V., et al., Comparison of  $^{15}\text{N}$  labelling methods to measure gross nitrogen mineralization, *Soil Biol. Biochem.* **31** (1999) 2015–2024.
- [30] SCHIMEL, D.S., Carbon and nitrogen turnover in adjacent grassland and cropland ecosystems, *Biogeochem.* **2** (1986) 345–357.
- [31] HATCH, D. J., et al., Nitrogen mineralization and microbial activity in permanent pastures amended with nitrogen fertilizer or dung, *Biol. Fert. Soils* **30** (2000) 288–293.
- [32] HART, S.C., et al., Dynamics of gross nitrogen transformations in an old-growth forest: the carbon connection, *Ecology* **75** (1994) 880–891.
- [33] PULLEMAN, M., TIETEMA, A., Microbial C and N transformations during drying and rewetting of coniferous forest floor material, *Soil Biol. Biochem.* **31** (1999) 275–285.
- [34] MURPHY, D.V., et al., Stratification of microbial biomass-C and N and gross N mineralization with soil depth in two contrasting Western Australian agricultural soils, *Aust. J. Soil Res.* **36** (1998) 45–55.
- [35] MURPHY, D.V., et al., Seasonal fluctuations in gross N mineralization, N consumption and microbial biomass in a Western Australian soil under different land use, *Aust. J. Agric. Res.* **49** (1998) 523–535.
- [36] RECOUS, S., et al., In situ changes in gross N transformations in bare soil after addition of straw, *Soil Biol. Biochem.* **31** (1999) 119–133.
- [37] RECOUS, S., et al., Concurrent measurements of soil N biotransformations using  $^{15}\text{N}$  dilution and modeling techniques, *Proceedings of the ASA-CSSA-SSSA Annual meeting*, Minneapolis, November 5–9 (2000).
- [38] KAY, B.D., “Soil structure and organic carbon: a review”, *Soil Processes and the Carbon Cycle* (LAL, R., et al., Eds.), CRC Press, Boca Raton (1998) 169–198.
- [39] LADD, J.N., et al., Simulation of  $^{14}\text{C}$  turnover through the microbial biomass in soils incubated with  $^{14}\text{C}$ -labelled plant residues, *Soil Biol. Biochem.* **27** (1995) 773–783.
- [40] AITA, C., Short term kinetics of residual wheat straw C and N under field conditions : characterisation by  $^{15}\text{N}^{13}\text{C}$  tracing and soil particle size fractionation, *Eur. J. Soil Sci.* **48** (1997) 283–294.
- [41] STRONG, D.T., “Soil architecture and its role in regulating gaseous emissions”, *Non- $\text{CO}_2$  Trace Gas Emissions  $\text{N}_2\text{O}$ ,  $\text{CH}_4$ ,  $\text{NO}$  from Belgian soils*, 24 October 2000, Ghent (in press).
- [42] CERRI, C., et al., Application du traçage isotopique naturel en  $^{13}\text{C}$  à l'étude de la dynamique de la matière organique des sols, *Comptes-rendus de l'Académie d'Agriculture des Sciences de Paris* **300** (1985) 423–428.
- [43] BALESSENT, J., et al., Relationship between soil organic matter dynamics to physical protection and tillage, *Soil Tillage Res.* **53** (2000) 215–230.
- [44] CHRISTENSEN, B.T., “Organic matter in soil-structure, function and turnover”, *DIAS Report Plant Production No 30*, Danish Institute of Agricultural Sciences, Foulum (2000).
- [45] TISDALL, J.M., OADES, J.M., Organic matter and water stable aggregates. *J. Soil Sci.* **33** (1982) 141–163.
- [46] ELLIOTT, E.T., Aggregate structure and carbon, nitrogen and phosphorus in native and cultivated soils, *Soil Sci. Soc. Am. J.* **50** (1986) 627–633.
- [47] BEARE, M.H., et al., Aggregate-protected and unprotected organic matter pools in conventionnal-tillage and no-tillage soils, *Soil Sci. Soc. Am. J.* **58** (1994) 787–795.
- [48] ANGERS, D.A., GIROUX, M., Recently deposited organic matter in soil water-stable aggregates, *Soil Sci. Soc. Am. J.* **60** (1996) 1547–1551.
- [49] SIX, J., et al., Aggregate and soil organic matter dynamics under conventional and no-tillage systems, *Soil Sci. Soc. Am. J.* **63** (1999) 1350–1358.

- [50] BALESDENT, J., et al., Effect of tillage on soil organic carbon mineralization estimated from  $^{13}\text{C}$  abundance in maize fields, *J. Soil Sci.* **41** (1990) 587–596.
- [51] GREGORICH, E.G., et al., Fertilization effects on soil organic matter turnover and corn residue C storage, *Soil Sci. Soc. Am. J.* **60** (1996) 472–476.
- [52] AOYAMA, M., et al., Particulate and mineral associated organic matter in water-stable aggregates as affected by mineral fertiliser and manure applications, *Can. J. Soil Sci.* **79** (1999) 419–425.
- [53] PUGET, P., et al., Total and young organic carbon distributions in aggregates of silty cultivated soils, *Eur. J. Soil Sci.* **46** (1996) 449–459.
- [54] SIX, J., et al., Soil structure and organic matter: I. Distribution of aggregate-size classes and aggregate-associated carbon, *Soil Sci. Soc. Am. J.* **64** (2000) 681–689.
- [55] SIX, J., et al., A re-evaluation of the enriched labile soil organic matter fraction, *Eur. J. Soil Sci.* **51** (2000) 283–293.
- [56] ANGERS, D., CHENU, C., “Dynamics of of soil aggregation and C sequestration”, *Soil Processes and the Carbon Cycle* (LAL, R., et al., Eds.), CRC Press, Boca Raton (1998) 199–206.
- [57] CHANTIGNY, M.H., et al., Soil aggregation and fungal and bacterial biomass under annual and perennial cropping systems, *Soil Sci. Soc. Am. J.* **61** (1997) 262–267.
- [58] GOLCHIN, J.A., et al., “A model linking organic matter decomposition, chemistry, and aggregate dynamics”, *Soil Processes and the Carbon Cycle* (LAL, R., et al., Eds.), CRC Press, Boca Raton (1998) 245–266.
- [59] MAGID, J., et al., In search of the elusive ‘active’ fraction of soil organic matter: three size-density fractionation methods for tracing the fate of homogeneously  $^{14}\text{C}$ -labelled plant materials, *Soil Biol. Biochem.* (1996) **28** 89–99.
- [60] LADD, J.N., et al., Carbon turnover and nitrogen transformations in an Alfisol and Vertisol amended with  $^{14}\text{C}$ -glucose and  $^{15}\text{N}$ - ammonium sulfate, *Soil Biol. Biochem.* **24** (1992) 359–371.
- [61] CHOTTE, J.L., et al., Sites of microbial assimilation, and turnover of soluble and particulate  $^{14}\text{C}$ -labelled substrates decomposing in a clay soil, *Soil Biol. Biochem.* **30** (1998) 205–218.
- [62] NICOLARDOT, B., et al., C and N fluxes between pools of soil organic matter: model calibration with long term incubation data, *Soil Biol. Biochem.* **26** (1994) 235–243.
- [63] JANS-HAMMERMEISTER, D.C., McGill, W.B., Evaluation of three simulation models used to describe plant residue decomposition in soil, *Ecological Modelling* **104** (1997) 1–13.
- [64] SMITH, P., et al., A comparison of the performance of nine soil organic models using datasets from seven long term experiments, *Geoderma* **81** (1997) 153–225.
- [65] McNEILL, A.M., et al., Use of in situ  $^{15}\text{N}$ -labelling to estimate the total below ground nitrogen of pasture legumes in intact soil-plant systems, *Aust. J. Agric. Res.* **48** (1997) 295–304.
- [66] ROCHETTE, P., et al., Separating soil respiration into plant and soil components using analyses of the natural abundance of carbon-13, *Soil Sci. Soc. Am. J.* **63** (1999) 1207–1213.

## EFFECT OF TILLAGE SYSTEM ON GROSS MINERALIZATION RATE OF A TYPIC ARGIUOLL SOIL (ARGENTINE)\*

A. PAZOS, C. VIDELA, H. ECHEVERRÍA, G. STUDDERT  
Facultad Ciencias Agrarias,  
Universidad Nacional de Mar del Plata, Balcarce, Argentina

P.C. TRIVELÍN  
Centro de Energia Nuclear na Agricultura,  
Universidade de São Paulo, Piracicaba, Brazil

### Abstract

A greenhouse experiment was carried out with the aim of determining the effects of tillage system on gross mineralization and nitrification rates. The soil used was a surface Typic Argiudoll under a) conventional tillage for 23 years (CT treatment), b) no tillage for 6 years (NT), and c) pasture for 4 years (P). Gross fluxes were measured with the  $^{15}\text{N}$ -dilution technique in PVC columns filled with 100-g amounts of soil. At 0, 7, 21 and 35 days, 3.5-mL aliquots of a solution of labelled  $(\text{NH}_4)_2\text{SO}_4$  (10%  $^{15}\text{N}$  a.e.) were injected at a rate of  $10 \mu\text{g N g}^{-1}$  soil. Twenty-four and 48 h after each injection, soil gravimetric water was determined, and ammonium and nitrate contents, and  $^{15}\text{N}$  enrichments of inorganic pools, were measured. Gross mineralization (GMR) and nitrification rates (GNR) were calculated. Pasture GMR was higher than those of the agricultural management treatments. Initial GMR of NT was high, but decreased rapidly, and CT presented continuously low GMRs. Pasture GMRs showed two peaks: higher initially, and another at day 35. This indicates the possible presence of two mineralizing pools. Gross nitrification rates were lower than GMRs showing that nitrifiers could be in competition with other ammonium consumers.

### 1. INTRODUCTION

Soil organic matter (SOM) decomposition and corresponding nitrogen (N) mineralization and immobilization are key processes of the N cycle in the soil-plant system. These processes are complex due to the heterogeneous components of SOM: from plant and animal residues, recently incorporated, to stable humic materials [1]. The biomass involved in decomposition is a mixture of microorganisms that are affected by the soil, environmental conditions—mainly soil temperature and water content—as well as by soil pH and energy status.

Agricultural soils of southeastern Buenos Aires province, Argentina, have high organic matter levels, between 25 and 45 g organic C  $\text{kg}^{-1}$ . Over the past two decades they have been continuously cropped under conventional tillage systems without pastures in the rotation [2]. Thus, their physical, chemical and biochemical properties have been degraded [3]. This degradation has resulted in erosion and nutrient deficiencies, mainly of N for wheat [4,5] and maize [6]. It has been shown organic matter content diminished by approximately 4.5 g organic C  $\text{kg}^{-1}$  after 6 to 7 years of conventional cropping, and reached equilibrium values between 26 and 29 g organic C  $\text{kg}^{-1}$  [7]. This indicates the presence of a very stable organic fraction, able to resist the disturbance caused by cropping. It must be a physically protected fraction, unavailable for mineralization, consequently, crops show nutrient deficiencies even though the SOM is conserved at a relatively high value.

Tillage system affects the physical, chemical, and biological properties of soil and determines crop-residue location and distribution. In turn, these alterations influence the characteristics and distribution of biological niches, affecting N-transformation processes and N-use efficiency by crops. Management practices that diminish soil disturbance are increasingly being adopted in Argentina. No-tillage management reached 7 Mha in 1999 (32% of the arable area) [8], contributing to diminished erosion and to conservation of organic matter. Consequently, nutrient dynamics under this system are now of great interest. No-tillage generally increases the amount of active soil N, as well as the C and N contents of microbial biomass, producing a stratification of fractions [9].

---

\*Work performed within the framework of projects funded by the Universidad Nacional Mar del Plata (15/A107) and Fundación Antorchas (13816-5).

Pasture inclusion in the rotation promotes the recuperation of degraded soils. It has been shown in southeastern Buenos Aires province that a 3- or 4-year pasture after a conventional cropping cycle of 6 to 7 years increased the organic matter to levels close to the original [7]. However, at the beginning of the pasture period, increases in availability of soil N are likely, due to contributions of labile fractions by plant roots.

Net changes in soil mineral N have been used as indicators of soil-N availability in situ [10,11]. Those changes are the result of combinations of gross processes that make up the N cycle, particularly ammonification, immobilization, and nitrification. One model [12] for estimating mineralized N has demonstrated a utility for evaluating the relative capacity of a soil to provide N to crops. However, that model presents difficulties when used independently of other variables that influence N dynamics, such as the presence of decomposing plant material—depending on its quality, amount, and physical form—and those related to soil management [13]. In recent years, studies using  $^{15}\text{N}$  have demonstrated that gross mineralization and immobilization, which determine N availability, are partially independent of each other [14]. Soil gross-N flow estimation allows a better understanding of processes governing the N cycle, and, therefore, the N availability for plants. Also, it facilitates the prediction of net mineralization. The  $^{15}\text{N}$ -dilution technique is the only tool for estimating gross-N flows unconfounded by the processes that consume  $\text{NH}_4^+$ , and it has been frequently used in recent years [15–17]. It is based on the decline of  $^{15}\text{N}$  abundance of the  $\text{NH}_4^+$  pool, initially labelled with  $^{15}\text{N}$ , by unlabelled  $\text{NH}_4^+$  that comes from mineralization of organic matter [18,19].

The aim of the present greenhouse experiment was to determine the effect of conventional and no-tillage systems on gross mineralization (GMR) and nitrification rates (GMR) and to compare them with those for soil under pasture, in the Pampas region of Argentina.

## 2. MATERIALS AND METHODS

The soil was a fine, mixed, thermic Typic Argiudoll collected at the Balcarce Experiment Station of the National Institute of Agricultural Technology (INTA) at Balcarce ( $37^{\circ}45'\text{S}$ ,  $58^{\circ}18'\text{W}$ , 130 m above sea level, 870 mm mean annual rainfall,  $13.7^{\circ}\text{C}$  mean annual temperature), Buenos Aires, Argentina. The surface horizon of this soil has a pH of 5.8, loamy texture,  $33.1\text{ cmol}_c\cdot\text{kg}^{-1}$  cation-exchange capacity, and  $43\text{ mg}\cdot\text{kg}^{-1}$  Bray and Kurtz P (after yearly fertilization). The experimental field had been under crop rotation for the previous 23 years. The treatments analysed were: a) conventional tillage for 23 years (CT), b) no tillage during the past 6 years (NT), and c) pasture for the past 4 years (P). A laboratory experiment was performed ( $22^{\circ}\text{C}$ ), packing 100-g soil samples (air dried and sieved through 2 mm) into PVC cylinders of 7-cm diameter and 5-cm height. The soil in the cylinders was moistened to field capacity, and moisture loss then prevented with a Parafilm cover with four pinholes to allow gas exchange. There were four replications by treatment and injection time. Ammonium sulphate, with an  $^{15}\text{N}$  excess of 10% was injected at 0, 7, 21 and 35 days at a rate of  $10\text{ }\mu\text{g N g}^{-1}$  of soil, using a device with seven syringes and hypodermic needles in a total volume of a 3.5 mL of solution. Twenty-four ( $t_0$ ) and 48 h ( $t_f$ ) after each injection, 50 g of soil from each cylinder were extracted with 200 mL KCl 1 M, shaken for 30 min. and filtered (Whatman GF/A glass microfibre filter papers).

Ammonium-N and  $\text{NO}_3^-$ -N contents of extracts were determined after steam distillation in the presence of MgO and Devarda's alloy [2]. Cross-contamination was prevented by distilling 25-mL aliquots of ethanol between samples and passing steam through the apparatus without condenser cooling [21]. The extracts were prepared for measurement of  $^{15}\text{N}$  enrichment by microdiffusion of 50 mL of extract with MgO over 4 days in 125-mL containers and another 3 days with Devarda's alloy [20]. Ammonia yielded by this procedure was trapped in small glass microfibre discs (Wathman GF/D), acidified with 10  $\mu\text{L}$  of 2.5 M  $\text{KHSO}_4$ , and enclosed in polytetrafluoroethylene tape (PTFE) [22]. After diffusion, the filters were packed in tin capsules and analysed for  $^{15}\text{N}/^{14}\text{N}$  isotope ratios using a mass spectrometer (Europa Sc. ANCA-NT) at the CENA-USP laboratory.



The gross mineralization rate was calculated using the classical isotope-dilution equation [23] following procedure B, because ammonium was only from the labelled pool:

$$A^*_t = \frac{A^*_0}{\left(1 + \frac{\theta \times t^m}{N_0}\right)^\theta}$$

where

$A^*_t$  is the atom % excess of the ammonium pool at time  $t$ ,

$A^*_0$  is the atom % excess of the ammonium pool at  $t=0$ ,

$\theta$  is  $(N_t - N_0)/t$  ( $\text{mg N kg}^{-1} \text{d}^{-1}$ ),

and  $m$  is the mineralization rate ( $\text{mg N kg}^{-1} \text{d}^{-1}$ ).

The ammonium mean pool  $^{15}\text{N}$  excess abundance was calculated with the equation:

$$[A^*]_{t_1-t_0} = \int A^*_t \frac{dt}{(t_1 - t_0)}$$

in order to estimate the gross nitrification rate, with the mineralization rate equation, but using the sizes and enrichments of the nitrate pool.

The results were analysed with analysis of variance and Tukey's test of mean differences, with previous verification of the fulfilment of assumptions of analysis of variance (variance normality and homogeneity).

### 3. RESULTS

The range of values for GMRs were similar to those reported before [24,25], although comparisons are difficult due to differences in soil characteristics and experimental conditions. At the second and third injection times (7 and 21 days), the changes in  $\text{NH}_4^+$  pool size were sometimes negative, resulting in small negative rates (which are not possible for GMRs), with large error terms.

Pasture GMR values were higher than those for agricultural management treatments (Table I). Initial GMR of NT was high, but decreased rapidly, and CT presented continuously low GMRs. Pasture GMR showed two peaks: a higher initially and another at day 35. This suggests the presence of two mineralizing pools.

The organic C under pasture was 5.2 and 7.7% higher than under NT and CT, respectively, although differences were not statistically significant. It has been demonstrated [26] that  $N_0$  (potentially mineralizable N) is more strongly correlated with the light organic matter fraction than with the total organic content. Also it has been found [27] that the macro-organic matter (a component of the light organic matter fraction in grassland soils) can be a significant source of mineralized N, and it may act also as a significant sink for mineral N. From our results, we may hypothesize that P, and possibly NT too, increased the light organic matter fraction with respect to CT, since this fraction is highly labile and can change rapidly in response to tillage [28]. Conservation treatments (P and NT) could be associated with higher mineralization rates.

For the same Balcarce experiment, the N derived from soil assimilated by wheat during the initial growing stages was greater under CT than under NT. However, these differences eventually disappeared (M.A. Melaj, personal communication). From these results it may be inferred that the higher initial values of soil N absorption under CT are the result of soil-aggregate disruption by tillage operations that caused physically protected pools of organic C and N to become available for microbial degradation.

TABLE I. GROSS MINERALIZATION AND NITRIFICATION RATES OF A TYPIC ARGIUOLL UNDER CONVENTIONAL TILLAGE (CT), NO TILLAGE (NT) AND PASTURE (P) MANAGEMENT SYSTEMS

Management system	Incubation (days)			
	0	7	21	35
Gross mineralization rate (mg·kg <sup>-1</sup> ·day <sup>-1</sup> )				
P	2.87(1.26) <sup>a</sup>	1.25(0.53)	0.42(0.08)	0.88(0.28)
CT	0.89(0.60)	0.25(0.58)	0.05(0.31)	0.25(0.26)
NT	1.87(0.42)	0.96(0.33)	0.22(0.58)	0.16(0.17)
Gross nitrification rate (mg·kg <sup>-1</sup> ·day <sup>-1</sup> )				
P	0.56(0.16)	0.44(0.17)	0.13(0.11)	0.14(0.06)
CT	0.52(0.11)	0.37(0.11)	0.29(0.12)	0.12(0.07)
NT	0.33(0.10)	0.27(0.13)	0.28(0.17)	0.09(0.04)

<sup>a</sup>Standard deviation.

Later, this effect would disappear, because after several years of continuous tilling under CT, only more recalcitrant soil organic fractions would remain. Under NT, however, the labile fraction accumulation, accompanied by lower temperatures, would have caused slower but continuous mineralization during the crop cycle. Gross nitrification rates were lower than GMRs (Table I) showing that nitrifiers may be in competition with ammonium consumers. Using aerobic incubation methodology [29], it was concluded that nitrification rate was dependent of ammonium production rate from organic matter in the same Balcarce soil. Present results show that, actually, ammonium production was not the limiting step for nitrification. At the first injection time, GNR under CT was higher than that under NT, but decreased to the same level after 36 days.

#### 4. CONCLUSIONS

The isotope-dilution technique allows the determination of GMRs for various management practices. Our results indicate that conservation treatments increase GMRs in comparison with soils continuously conventionally managed for long periods of time. Gross nitrification rates were lower than GMRs, showing an active competition for ammonium between nitrifying and immobilizing organisms that limits nitrate production.

#### REFERENCES

- [1] JENKINSON, D.S., "The turnover of organic matter in soil", Soil conditions and Plant Growth (WILD, A., Ed.), Longmans, London (1988) 564–607.
- [2] DARWICH, N.A., "Estado actual y manejo de los recursos naturales en la región pampeana húmeda sur", Seminario Juicio a Nuestra Agricultura, Hacia el Desarrollo de una Agricultura Sostenible, Actas Seminario Instituto Nacional de Tecnología Agropecuaria, Buenos Aires (1991) 51–62.
- [3] ECHEVERRÍA, H.E., FERRARI, J.L., Relevamiento de Algunas Características de los Suelos Agrícolas del Sudeste Bonaerense, Boletín Técnico No. 112, INTA, CeRBAS, EEA, Balcarce (1993).
- [4] BERARDO, A., Aspectos Generales de Fertilización y Manejo del Trigo en el Area de Influencia de la Estación Experimental INTA-Balcarce, Boletín Técnico No. 28, EEA, INTA, Balcarce (1994).
- [5] ECHEVERRÍA, H.E., VIDELO, C.C., Eficiencia fisiológica y de utilización de nitrógeno en trigo en la Región Pampeana argentina, Ciencia del Suelo **16** (1998) 83–87.

- [6] ANDRADE, F.H., et al., “Requerimientos de nutrientes minerales”, Bases para el Manejo del Maíz, el Girasol y la Soja (ANDRADE, F.H., SADRAS, V.O., Ed.), EEA INTA Balcarce-Fac. de Ciencias Agrarias UNMP (2000).
- [7] STUDDERT, G.A., et al., Crop-pasture rotation for sustaining the quality and productivity on a Typic Argiudoll, *Soil Sci. Soc. Am. J.* **61** (1997) 1466–1472.
- [8] AAPRESID, Superficie de Trigo en Siembra Directa, Jornadas de Intercambio Técnico, AAPRESID, Mar del Plata (1999).
- [9] DORAN, J.W., Microbial biomass and mineralizable nitrogen distributions in no-tillage and plowed soils, *Biol. Fert. Soils* **5** (1987) 68–75.
- [10] HATCH, D.J., et al., Field measurement of nitrogen mineralization using soil core incubation and acetylene inhibition of nitrification, *Plant Soil* **124** (1990) 97–107.
- [11] STENGER, R., et al., Rates of net nitrogen mineralization in disturbed and undisturbed soils, *Plant Soil* **171** (1995) 323–332.
- [12] ECHEVERRÍA, H.E., et al., Un modelo para estimar la mineralización de nitrógeno en suelos del sudeste de la provincia de Buenos Aires (Argentina), *Ciencia Suelo* **12** (1994) 56–62.
- [13] STUDDERT, G.A., ECHEVERRÍA, H.E., Crop rotations and nitrogen fertilization to manage soil organic carbon dynamics, *Soil Sci. Soc. Am. J.* **64** (2000) 1496–1503.
- [14] DAVIDSON, E.A., et al., Measuring gross mineralization, immobilization, and nitrification by  $^{15}\text{N}$  isotopic pool dilution in intact soil cores, *J. Soil Sci.* **42** (1991) 335–349.
- [15] BARRACLOUGH, D., PURI, G., The use of  $^{15}\text{N}$  pool dilution and enrichment to separate the heterotrophic and autotrophic pathways of nitrification, *Soil Biol. Biochem.* **27** (1995) 17–22.
- [16] SPARLING, G.P., et al., Short-term net mineralization from plant residues and gross and net N mineralization from soil organic matter after rewetting of a seasonally dry soil, *Aust. J. Soil Res.* **33** (1995) 961–973.
- [17] RECOUS, S., et al., In situ changes in gross N transformations in bare soil after addition of straw, *Soil Biol. Biochem.* **31** (1999) 119–133.
- [18] KIRKHAM, D., BARTHOLOMEW, W.V., Equations for following nutrient transformations in soil, utilising tracer data, *Soil Sci. Soc. Am. Proc.* **18** (1954) 33–34.
- [19] KIRKHAM, D., BARTHOLOMEW, W.V., Equations for following nutrient transformations in soil, utilising tracer data: II, *Soil Sci. Soc. Am. Proc.* **19** (1955) 169–192.
- [20] BREMNER, J.M., “Inorganic forms of nitrogen”, *Methods of Soil Analysis, Part 2* (BLACK, C.A., Ed.), ASA, Madison (1965) 1149–1178.
- [21] PRUDEN, G., et al., The measurement of  $^{15}\text{N}$  in soil and plant material, *Fert. Res.* **6** (1985) 205–218.
- [22] SØRENSEN, P., JENSEN, E.S., Sequential diffusion of ammonium and nitrate from extracts to a polytetrafluoroethylene trap for  $^{15}\text{N}$  determination, *Anal. Chim. Acta* **252** (1991) 201–203.
- [23] BARRACLOUGH, D., The use of mean pool abundances to interpret  $^{15}\text{N}$  tracer experiments. I. Theory, *Plant Soil* **131** (1991) 89–96.
- [24] PILBEAM, C.J., WARREN, G.P., Use of  $^{15}\text{N}$  for fertilizer N recovery and N mineralization studies in semi-arid Kenya, *Fertilizer Res.* **42** (1995) 123–128.
- [25] PURI, G., ASHMAN, M.R., Relationship between soil microbial biomass and gross N mineralization, *Soil Biol. Biochem.* **30** (1998) 251–256.
- [26] CURTIN, D., WEN, G., Organic matter fractions contributing to soil nitrogen mineralization potential, *Soil Sci. Soc. Am. J.* **63** (1999) 410–415.
- [27] MONAGHAN, R., BARRACLOUGH, D., Contributions to gross N mineralization from  $^{15}\text{N}$ -labelled soil macroorganic matter fractions during laboratory incubation, *Soil Biol. Biochem.* **27** (1995) 1623–1628.
- [28] JANZEN, H.H., et al., Light-fraction organic matter in soils from long-term rotations, *Soil Sci. Soc. Am. J.* **56** (1992) 1799–1806.
- [29] Navarro, C.A., et al., “Cinética de las reacciones de amonificación y nitrificación en algunos suelos de la Argentina”, *Actas IX Reunión Argentina de la Ciencia del Suelo, Paraná, Tomo II, Asociación Argentina de la Ciencia del Suelo, Buenos Aires* (1980) 431–437.

## USE OF NATURAL ABUNDANCE MEASUREMENTS OF $^{15}\text{N}$ IN RESIDUAL NITRATE ASSESSING DENITRIFICATION IN BUFFER STRIPS

K. DHONDT, P. BOECKX, O. VAN CLEEMPUT, G. HOFMAN

Faculty of Agricultural and Applied Biological Sciences,  
Ghent University, Ghent, Belgium

The restoration or construction of riparian buffer strips could be an effective option to reduce diffuse pollutant input (especially  $\text{NO}_3^-$ ) into streams caused by nutrient loss from agriculture. Nitrate removal and its relation to  $^{15}\text{N}$  isotopic fractionation were evaluated in a riparian buffer strip in Velzeke-Ruddershove, Belgium.

Groundwater was sampled at the beginning of April. Several transects with piezometers were installed parallel to the slope of the topography of the buffer strip. Analyses of  $\text{NO}_3^-$  concentrations indicated that  $\text{NO}_3^-$  was lost extremely rapidly from shallow groundwater that passed through the buffer zone. Nitrate concentrations decreased approximately 100% over a distance of 6 m.

Isotopic analysis of the  $\text{NO}_3^-$  samples was conducted using an ANCA-TGII coupled to an isotope ratio mass spectrometer (20-20, PDZ Europa) after conversion of  $\text{NO}_3^-$  to nitrous oxide ( $\text{N}_2\text{O}$ ) by cadmium reduction. As  $\text{NO}_3^-$  concentrations in the shallow groundwater (<2 m) decreased during its flow through the riparian buffer zone, progressive enrichment in  $^{15}\text{N}$ - $\text{NO}_3^-$  and, consequently, increased delta values ( $\delta^{15}\text{N}$ ) were observed (Fig. 1). This trend suggests that denitrification was at least partly responsible for  $\text{NO}_3^-$  removal. Plant uptake was still limited during this period of the year and it is assumed that no  $^{15}\text{N}$  enrichment was associated with  $\text{NO}_3^-$  uptake [1].

At the input side of the buffer strip,  $\delta^{15}\text{N}$  values of +15‰ were measured, indicating that animal manure, applied on adjacent agricultural fields was the chief source of  $\text{NO}_3^-$  in the groundwater. This result is in accordance with a fertilizer-use survey.

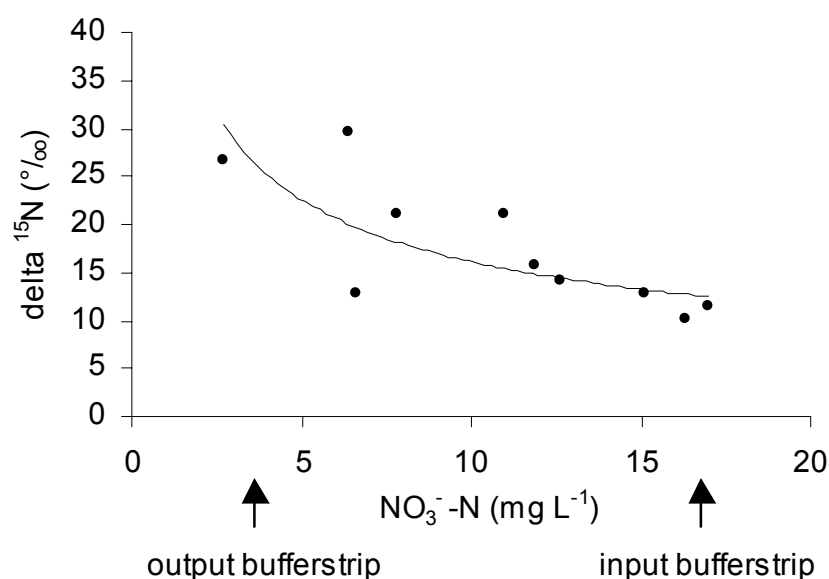


FIG. 1. Relationship between nitrate concentrations in shallow ground water and  $\delta^{15}\text{N}$  values.

In order to define the specific isotopic enrichment due to denitrification in the riparian zone, the following laboratory experiment was set up. Surface soil (0–10 cm, 585 g) was placed in a 2-L flask. A solution of 1 L containing 28 mg  $\text{NO}_3^-$ -N was added to the soil. Nitrapyrin was used as a nitrification inhibitor, at a rate of 120 mg  $\text{kg}^{-1}$  dry soil. To promote denitrification, 20 g finely ground wheat straw was added as a C source. The bottle was sealed with a rubber stopper having holes fitted with glass stopcocks and continuously flushed with He. The soil suspension was incubated at 25°C and continuously stirred. Samples were taken at regular time intervals and analysed for  $\text{NO}_3^-$  and  $\delta^{15}\text{N}$ - $\text{NO}_3^-$ .

A clear enrichment of  $^{15}\text{N}$  was observed as the  $\text{NO}_3^-$  concentrations in the soil slurry decreased. This increase in  $\delta^{15}\text{N}$  values corresponded to a denitrification enrichment factor ( $\epsilon$ ) of –64‰. This value is higher than those reported before (–17 to –29‰) [1, 2]. To assess the  $\text{NO}_3^-$  losses attributed to denitrification in the riparian zone, additional field sampling (see Fig. 1) should be carried out. This is necessary to determine reliable  $\epsilon$  values under field conditions. In the future, it will also be investigated whether declining  $\text{NO}_3^-$  concentrations are due to dilution by deeper ground water. Therefore, measurements of  $\text{NO}_3^-:\text{Cl}^-$  ratios will be conducted.

## REFERENCES

- [1] LUND, L.J., et al., Estimating denitrification in a large constructed wetland using stable nitrogen isotope ratios, *Ecol. Eng.* **14** (2000) 67–76.
- [2] MARIOTTI, A., et al., Experimental determination of nitrogen kinetic isotope fractionation: some principles; illustration for the denitrification and nitrification processes, *Plant Soil* **62** (1981) 413–430.

## SOYBEAN BENEFIT TO A SUBSEQUENT WHEAT CROP IN A CROPPING SYSTEM UNDER ZERO TILLAGE\*

B.J.R. ALVES, L. ZOTARELLI, R.M. BODDEY, S. URQUIAGA,  
EMBRAPA Agrobiologia,  
Seropédica – RJ, Brazil

### Abstract

This experiment was conducted to compare biological nitrogen fixation (BNF) contributions to soybean crops and the effect of the presence of its residues to the production of wheat under two tillage systems. The plots were established in the experimental area of the Embrapa Soybean Centre in the State of Paraná, southern Brazil. A soybean/wheat rotation was planted under conventional and zero tillage (CT and ZT, respectively). The contribution of BNF to soybean was assessed using the  $\delta^{15}\text{N}$  technique and the relative abundance of ureides. An N balance was performed based on the dry matter and N content of, and the BNF contribution to, the whole crop. Plant-residue decomposition was evaluated by field sampling. Wheat responses to the previous soybean crop and tillage treatments were evaluated from the wheat yields and the 'A'-value technique. Comparing ZT and CT, there were no marked differences in grain yield or N accumulation by the crops, but BNF was higher in the soybean under ZT. Although for both tillage systems the BNF contribution to soybean was over 170 kg N/ha, the benefit to the subsequent crop was due to the release of N from extremely labile soybean residues of low C:N ratio and not because of a net gain of N from BNF.

### 1. INTRODUCTION

The area in Brazil cropped with zero tillage (ZT) has been increasing rapidly since 1995, and in the southern region it has been adopted on over 50% of the area under crop production [1]. This is explained by the advantages that it offers in terms of protection of the soil against erosion, but mainly by economic benefits due to reduction of mechanical field operations [2].

In order to optimize the N economy in ZT, the amount and quality of biomass are considered in the choice of crops for the rotation. Biological nitrogen fixation (BNF) represents a free source of N and legumes are considered to introduce N through crop residues. Soybean is one of the most important grain-legume crops in the summer, and its successful production depends ultimately upon adequate functioning of the  $\text{N}_2$ -fixing symbiosis. Also, since the sustainability of any production system requires a balance of nutrient inputs and outputs [3], low rates of BNF to soybean will create a dependence on soil N-reserves resulting in an exportation of N from the soil as soybean usually presents a high N-harvest index. In Brazil, it is almost a rule that any crop succeeding soybean has its productivity improved. However, the nature of the contribution of soybean to the succeeding crop remains uncertain.

In this paper we report the influence of tillage on the contribution of BNF to soybean, assessed by the  $^{15}\text{N}$  natural-abundance and ureide-abundance techniques and the residual effects of the soybean crop on soil N availability and wheat yields.

### 2. MATERIAL AND METHODS

#### 2.1. Site description

The study was carried out in the south of Brazil in the experimental area of the Embrapa Soybean Centre sited near Londrina, northern Paraná State. Soybean (*Glycine max*) is planted in the summer months, October to December, and is harvested from late January to March. The climate is humid sub-tropical with monthly minimum and maximum temperature means in the range of 12 to 27°C and a mean rainfall of about 800 mm during this crop. The soil of this area is a dystrophic Haplorthox with

---

\*Work supported by IAEA contract 10953/RO.

71% clay and high iron saturation (dusky red latosol). It presented the following chemical characteristics in the 0- to 20-cm layer: pH (H<sub>2</sub>O) 5.6; 11 mg/kg of P (Mehlich I); K, Ca and Mg (cmol/dm<sup>3</sup>), 0.31, 5.4 and 3.0, respectively; C and N, 1.32 and 0.12%, respectively. Experimental plots were established in a randomized block design that comprised CT and ZT treatments. Conventional tillage consisted of ploughing three times, firstly with a mold-board plough and then twice with a disc plough. Each plot was 12×6 m with four replicates for each tillage treatment. The area was under zero tillage for 3 consecutive years before this experiment was installed, and a winter crop of black oats (*Avena strigosa*) had been grown immediately before.

## 2.2. BNF contribution to soybean

The soybean cultivar Embrapa BR 48 was sown after being treated with a commercial inoculant of *Bradyrhizobium japonicum* at 500 g of product per 60-kg aliquot of seeds. In addition, the seeds were treated with fungicides to avoid root diseases. Simultaneous with planting, the soil was fertilized with an equivalent of 220 kg/ha of a 0-20-20 NPK formulation. In each plot of soybean, a 1-m length of row had all shoot and root mass sampled at 56, 64, 78, 97, and 126 days after planting (DAP). Sampled shoot material was separated into leaves, stems plus petioles, pods, and seeds. Roots were sampled from a soil volume of 1.0×0.5×0.3 m, and separated from soil by sieving to 2 mm. Root nodules that passed through the sieve were handpicked. With the aim of measuring total N accumulation of soybean, a 0.5×1.0-m net was positioned between two rows to collect daily all senesced leaves. Estimation of the BNF contribution to soybean was based on the <sup>15</sup>N-abundance technique [4] and a control plant was sampled at all dates. The control plants were the weeds that encroached into the plots. All sampled plant material was oven-dried (65°C), weighed and milled to powder by the use of a prototype roll mill [5], except for a proportion of the stems and petioles of the soybean which was ground in a Wiley mill for N-solute analysis for application of the ureide-abundance technique. All of the powdered material was analysed for total N and <sup>15</sup>N natural abundance in a Finnigan Delta Plus mass spectrometer coupled to a Carlo Erba CN analyser (Model EA 1080). The formula used to quantify BNF to soybean (%Nd<sub>fa</sub>), using δ<sup>15</sup>N values of plant material was:

$$\%Nd_{fa} = \frac{(\delta^{15}N_{control} - \delta^{15}N_{legume})}{(\delta^{15}N_{control} - B)} \times 100$$

where

B is the discrimination of <sup>15</sup>N during BNF [4]; based on Refs. [6,7] a mean δ<sup>15</sup>N value of −1.00‰ was used for B.

Coarse-milled material of stems plus petioles of soybean were boiled in a water bath for 3 min and filtered as described by Peoples et al. [6]. Aliquots of the extracts were further analysed for ureides [6] and for nitrate using a flow-injection technique [8]. Relative abundance of ureides (RUA) was calculated for each sampling date using the expression:

$$RUA = \frac{4 \text{ nmol ureide}}{(4 \text{ nmol ureide} + \text{nmol nitrate})} \times 100$$

RUA data were transformed to %Nd<sub>fa</sub> using the following equations [9], for the vegetative stage:

$$\%Nd_{fa} = -27.2 + \frac{\sqrt{(0.0642 + 0.0228 \times RUA)}}{0.0114}$$

and for the reproductive stage:

$$\%Nd_{fa} = -73.5 + \frac{\sqrt{(0.1045 + 0.0136 \times RUA)}}{0.0068}$$

### 2.3. Decomposition of plant residues

Immediately before sowing the soybean, residues of the previous oat crop on the soil surface were sampled in four replicates from areas of 0.25 m<sup>2</sup> in each plot under ZT. Residues on ZT plots were sampled again at 72 and 148 DAP. At 148 DAP, the residues under the 0.5 m<sup>2</sup> net (for fallen leaves) were also sampled. A last sampling of soil-surface residues was made just before the wheat harvest. All sampled plant material was oven-dried (65°C), weighed and pulverized in a Wiley mill for total N analysis.

### 2.4. Soil 'A'-value

The 'A'-value technique [10] was employed to evaluate the effect of the soybean residues on availability of soil N to wheat (*Triticum aestivum*) cv. Ocepar 18. In this case, an area beside the main plots under ZT microplots was kept fallow during the summer and sown to wheat. In one of these microplots, the surface residues were removed. Inside of each main plot previously planted with soybean under ZT, two more treatments were introduced. In one of them, surface residues were left intact, and, in the other, surface residues were removed. In the CT area in one microplot, the soil-surface residues were incorporated. All plots and microplots were fertilized with 14 kg P/ha of P and 23 kg K/ha. Nitrogen was applied as a split dose as 20 kg/ha at planting and 20 kg/ha at 30 DAP, and, in the case of microplots, the same quantity of ammonium sulphate enriched with 1 atom %<sup>15</sup>N i excess (%<sup>15</sup>N xs) was applied instead of the unlabelled fertilizer. At harvest, wheat plants in the microplots were sampled, oven-dried (65°C), weighed, and milled, as the soybean material for total N and <sup>15</sup>N analysis. The 'A'-value was calculated as follows:

$$'A'-value = \frac{(\%Ndfs \times Nf)}{\%Ndff}$$

where

%Ndfs is the percentage of N in the wheat plants derived from soil

$$\%Ndfs = \left(1 - \frac{plant \%^{15}N xs}{fertilizer \%^{15}N xs}\right) \times 100$$

%Ndff is the percentage of N in the wheat plants derived from fertilizer

$$\%Ndff = \frac{plant \%^{15}N xs}{fertilizer \%^{15}N xs} \times 100$$

and Nf is the total N added as fertilizer (kg/ha).

## 3. RESULTS AND DISCUSSION

The dry-matter accumulation by soybean in the first 56 days of growth under ZT was 47% lower than under CT (Fig. 1), which could be a result of soil compaction that was observed in the surface 10 cm under ZT. Plant roots in ZT grew laterally after reaching a depth of 8 cm and then changed direction again to deeper layers. This stress was the best explanation for the delayed initial plant development. Soybean-leaf drop began after 72 DAP and was accentuated during the pod-fill stage. At harvest, this amounted 1.5 t/ha of dry matter with 24.6 kg N/ha in CT, and 1.2 t/ha of dry matter with 22.3 kg N/ha in ZT.

Tillage practice affected neither the final accumulation of total plant dry mass nor N. The mean grain yield of soybean was 2.9 t/ha under ZT and 3.0 under CT, with an accumulation in the grain of 175 kg N/ha under ZT and 182 under CT (Table I).



TABLE I. SOYBEAN GRAIN YIELD, RESIDUE DRY MATTER AND N ACCUMULATION, AND BNF UNDER ZT AND CT

Tillage system	Grain yield	Residue <sup>a</sup>	Grain N	Residue N	Plant N	BNF	N balance <sup>b</sup>
	— (t/ha) —				(kg/ha)		
CT	3.03	3.23	182	38.1	219	158	−23.4
ZT	2.91	3.09	175	31.4	206	169	−5.70
t-Test	ns	ns	ns	ns	ns	*	*
CV(%)	35.5	20.5	32.7	14.8	30.1	—	—

<sup>a</sup>Except grain, all plant dry matter including recoverable roots and senesced leaves.

<sup>b</sup>BNF (whole plant) minus N in grain.

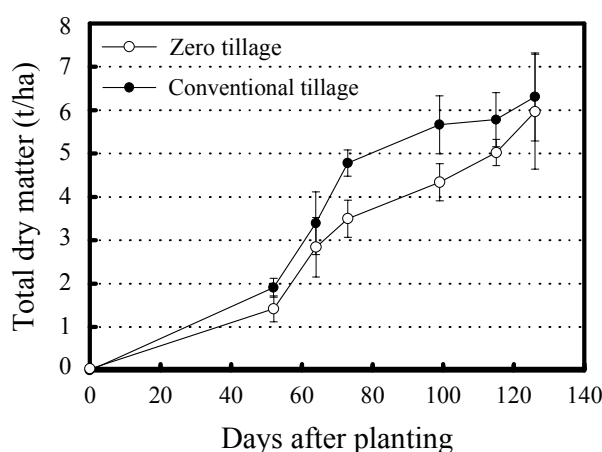


FIG. 1. Accumulation of dry matter by soybean cultivated under conventional tillage and zero tillage. Bars indicate standard error of means of four replicates.

The two techniques employed to measure the contribution of BNF to soybean gave estimates that were in good agreement. The  $\delta^{15}\text{N}$  values obtained for the weeds varied from 7.5 to 7.7 in the experimental area and the mean values obtained for soybean under ZT was 0.3 and under CT 1.4, which resulted in estimates of BNF of 83% to the plants under ZT and of 71% in the case of CT.

Estimates of BNF contribution using ureide abundance (RUA) were 80% under ZT to 72% under CT. The lower BNF contribution observed in CT can be attributed to positive effects of mechanical disturbance on soil N mineralization. It is well known that soil disturbance induces a flush of mineral N due to attack of soil microbes on labile organic matter exposed by disruption of soil aggregates [11]. Hungria et al. [12] pointed out that, in areas cultivated for the first time, nodulation may not be effective due to high concentrations of N mineral. Suppressive effect of soil nitrate on nodulation and BNF were observed also by Herridge and Betts [13]. In the present study, soybean nodule dry weight under CT was 40% lower than under ZT at the first sampling date (56 DAP).

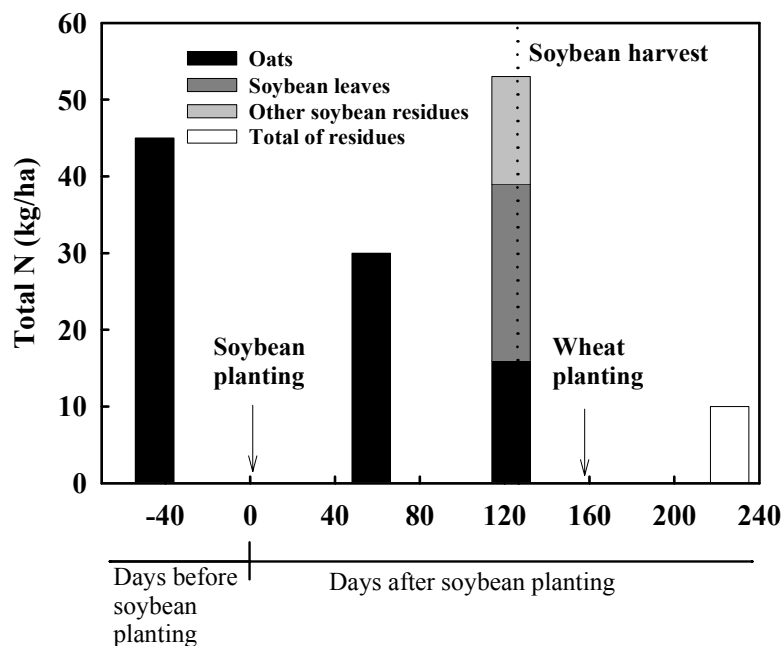


FIG. 2. Decomposition of plant residues on the surface of plots under zero tillage during soybean and wheat cultivation.

For the N balance of the soybean crop, the difference between the BNF input and the N exported in grain gave a negative value, estimated at  $-6$  and  $-23$  kg N/ha for ZT and CT, respectively (Table I). Hughes and Herridge [14] found similar BNF contributions to soybean, but their N balances were positive because their cultivar presented a relatively low N-harvest index ( $\sim 59\%$ ), considerably less than the Brazilian cultivar used in this study ( $\sim 83\%$ ).

Forty-five days before soybean planting under ZT, the residues on the soil surface (mainly from black oats) contained 4.1 t/ha of dry matter and 45 kg N/ha. At the second sampling (57 days after soybean planting), the residues were reduced to 2.9 t/ha and 30 kg N/ha (Fig. 2). Leaves of soybean began senescing and falling from this date, and at the sampling immediately before soybean harvest, residues contained 2.3 t/ha of dry mass with 39 kg N/ha. The amount of residues of oats under the net used for the collection of soybean leaves was 1.3 t/ha which suggests that part of the 200 kg/ha of residues belonging to oats decomposed more quickly as a result of a synergistic effect of soybean residues. The amount of N in the residues after soybean harvest was approximately 53 kg/ha, and after 143 days, at the time of the wheat harvest, total N in residues decreased to only  $\sim 10$  kg/ha.

Where wheat followed soybean, cereal grain yield reached 2.4 t/ha under both ZT and CT, approximately 30% greater than when it followed a summer fallow (Table II). Incorporation of oat residues did not improve wheat production. Removing surface residues from the microplots of soybean main plots did not reduce wheat production since the labile fraction of these residues, principally that from soybean, had already decomposed and the N liberated to the soil. As the N balance of soybean was almost nil or negative, it could be deduced that the rapid mineralization of the N of the soybean residues favoured wheat growth by increasing soil-N availability [15]. An effect of the quality of legume residue was also reported by Senaratne and Hardarson [16] and, using the 'A'-value technique, they estimated that a pea-sorghum crop sequence promoted an increment of 74 kg/ha in soil-N availability in comparison to a barley-sorghum sequence. In the present experiment, the 'A'-value technique indicated that the increase in N availability to the wheat crop after soybean (approximately 29 kg N/ha) was very close to the amount of N in the senesced leaves of soybean (22.3 to 24.6 kg N/ha) plus the residues of this crop left after harvest (8.7 to 13.5 kg N/ha).

TABLE II. GRAIN YIELD, N ACCUMULATION AND INCREASE IN PLANT N AVAILABILITY OF THE SOIL PREVIOUSLY PLANTED WITH SOYBEAN OR LEFT IN FALLOW DURING THE SUMMER

Condition of wheat crop	Grain yield (t/ha)	Total N		Increase in soil available N <sup>a</sup> (kg/ha)
		Grain	Shoot	
		—— (kg/ha) ——		
Area previously planted with soybean				
With residues	2.46a <sup>b</sup>	56.7a	30.0ab	29.7a
Residues incorporated	2.31a	55.4a	22.6b	30.2a
Residues removed	2.22abc	52.2a	37.2a	23.5a
Fallow area				
With residues	1.77bc	41.0b	36.8a	4.3b
Residues removed	1.85bc	46.0 b	35.2 a	0.0b
C.V. (%)	16.3	14.9	20.3	7.4

<sup>a</sup>Estimated by the difference in 'A'-value of the soil in the area previously planted with soybean and the area under fallow. Ammonium sulphate (1 atm %15N excess ) was applied at a rate of 40 kg/ha of N.

<sup>b</sup>Means followed by the same letter in the same column are not significantly different ( $P < 0.05$ ).

#### 4. CONCLUSION

Comparing ZT and CT, there were no marked differences in grain yield or N accumulation by the crops, although BNF was higher in the soybean under ZT. In both tillage systems, the BNF contributions to soybean were over 170 kg N/ha, yet the benefit to the subsequent crop was due to release of N from the extremely labile soybean residues of low C:N ratio and not because of a net gain of N from BNF.

#### REFERENCES

- [1] ALVES, B.J.R., et al, "Ciclaje de N en sistemas de siembra directa y convencional", Jornada Sobre Biología del Suelo en Siembra Directa (Proc. Symp. Buenos Aires, 1999) (RIMOLO, M. M., Ed.), INTA, Buenos Aires (1999) 1–7.
- [2] DERPSCH, R., Historical Review of No-Tillage Cultivation of Crops, [http://www.ao.org/ag/AGS/AGSE/agse\\_e/3ero/harare/PartII/21Derp.htm](http://www.ao.org/ag/AGS/AGSE/agse_e/3ero/harare/PartII/21Derp.htm).
- [3] GREENLAND, D.J., Bringing the green revolution to the shifting cultivator, *Science* **190** (1975) 841–844.
- [4] SHEARER, G., KOHL, D.H., N<sub>2</sub>-fixation in field settings: estimations based on natural N abundance, *Aust. J. Plant Physiol.* **13** (1986) 699–756.
- [5] SMITH, J.L., MYUNG, H.U., Rapid procedures for preparing soil and KCl extracts for <sup>15</sup>N analysis, *Commun. Soil Sci. Plant Anal.* **21** (1990) 2273–2279.
- [6] PEOPLES, M.B., et al., Methods for Evaluating Nitrogen Fixation by Nodulated legumes in the Field, ACIAR, Canberra (1989).
- [7] BERGERSEN F.J., et al., Nitrogen fixation during vegetative and reproductive growth of irrigated soybeans in the field: Application of  $\delta^{15}\text{N}$  methods, *Aust. J. Agric. Res.* **43** (1992) 145–153.
- [8] ALVES, B.J.R., et al., Rapid and sensitive determination of nitrate in plant tissue using flow injection analysis, *Commun. Soil Sci. Plant Anal.* **31** (2000) 2739–2750.
- [9] HERRIDGE, D.F., PEOPLES, M.B., Ureide assay for measuring nitrogen fixation by nodulated soybean calibrated by <sup>15</sup>N methods, *Plant Physiol.* **93** (1990) 495–503.

- [10] FRIED, M., DEAN, L.A., A concept concerning the measurement of available soil nutrient, *Soil Sci.* **73** (1952) 263–271.
- [11] FRANZLUEBBERS, A.J., Potential C and N mineralization and microbial biomass from intact and increasingly disturbed soils of varying texture, *Soil Biol. Biochem.* **31** (1999) 1083–1090.
- [12] HUNGRIA, M., et al., Importância do Sistema de Semeadura Direta na População Microbiana do Solo, Série Documentos, EMBRAPA Soja, Londrina (1997).
- [13] HERRIDGE, D.F., BETTS, J.H., Field evaluation of soybean genotypes selected for enhanced capacity to nodulate and fix nitrogen in the presence of nitrate, *Plant Soil* **110** (1988) 129–135.
- [14] HUGHES, J.F., HERRIDGE, D.F., Effect of tillage on yield, nodulation and nitrogen fixation of soybeans in far north coastal New South Wales, *Aust. J. Expl. Agric.* **29** (1989) 671–677.
- [15] CHALK, P., Dynamics of biologically fixed N in legume-cereal rotations: a review, *Aust. J. Agric. Res.* **49** (1998) 303–316.
- [16] SENARATNE, R., HARDARSON, G., Estimation of residual N effect of faba bean and pea on two succeeding cereals using <sup>15</sup>N methodology, *Plant Soil* **110** (1988) 81–89.

## A $^{15}\text{N}$ POOL-DILUTION APPROACH TO MEASURE GROSS N-TRANSFORMATIONS FOLLOWING APPLICATIONS OF DOMESTIC SLUDGE AND COMPOST TO A FIELD SOIL

P. AMBUS, L.K. KURE  
Risø National Laboratory,  
Roskilde

E.S. JENSEN  
The Royal Veterinary and Agricultural University,  
Tåstrup

Denmark

Recycling industrial and domestic organic wastes onto soils is gaining interest as a means of achieving sustainable crop-nutrient management and of reducing the use of chemical fertilizers. In Denmark, about 50% of the sludge from sewage-treatment plants is used for agricultural purposes in order to improve soil quality and fertility by increasing the organic matter and nitrogen (N) contents.

Although the initial fertilizer value of waste material is associated with its inorganic N content, subsequent decomposition processes influence the availability of N in either the positive or negative direction. Assessing gross N turnover requires application of the stable isotope  $^{15}\text{N}$  and subsequent analysis of the  $^{14}\text{N}$  and  $^{15}\text{N}$  pool sizes. Such approaches are relatively resource-demanding and have been undertaken in only a limited number of field experiments.

In this study, gross N-turnover rates were followed for 1 year by examining  $^{15}\text{N}$  pool dilution in a field experiment with controlled application of anaerobically treated sewage sludge (424 g DM  $\text{m}^{-2}$ , 2.9% N) and composted household waste (1,784 g DM  $\text{m}^{-2}$ , 1.7% N). The waste materials were mixed into the soil in field microplots confined by open-ended 10-cm-diameter PVC rings. Gross mineralization and immobilization rates were measured on seven occasions at weekly to monthly intervals. The top 15 cm of soil in sextuple sets of microplots were uniformly labelled with a  $^{15}\text{NH}_4^{15}\text{NO}_3$  (50:50 10%  $^{15}\text{N}$  excess) solution, and the change in  $^{15}\text{N}$  enrichment and total inorganic N ( $\text{N}_i$ ) measured over a 48-h incubation period. Gross N-mineralization rates were calculated using the equations given in Ref. [1]. Soil samples were rinsed carefully with KCl in order to achieve  $^{15}\text{N}$  immobilized in the organic fraction [2].

Gross N-mineralization showed distinct seasonal patterns for all the treatments, with maximum rates during the summer (Fig. 1), possibly associated with higher soil temperatures. In the initial period during spring with both waste materials and during summer and early autumn with compost, gross N-mineralization tended to be higher than in the control soil. However, neither of the waste treatments were statistically significant on a time-point basis due to great variability among the replicate microplots. Soil  $\text{N}_i$  availability increased markedly with the sludge and compost treatments. The increase was most pronounced with sludge, in which  $\text{N}_i$  remained 113% higher than in control soil throughout the summer. Although the N addition with compost was 2.5-fold greater than with sludge, compost additions showed only an initial transient increase in soil  $\text{N}_i$  (day 0 and day 14), and after four weeks of incubation there was no further detectable effect on  $\text{N}_i$ .

The results indicate that anaerobically treated sewage sludge has a relatively high fertilizer value that extends over a growing season, despite minor effects on the soil gross N-mineralization. Household compost, on the other hand, has relatively little fertilizer value although it tended to increase N mineralization more than did sludge. The contrasting data with respect to mineralization and  $\text{N}_i$  release may result from different responses in the gross N-immobilization following the waste applications, as a result of differing substrate quality. This statement needs verification pending data analysis from the field experiment and chemical analysis of the waste materials.

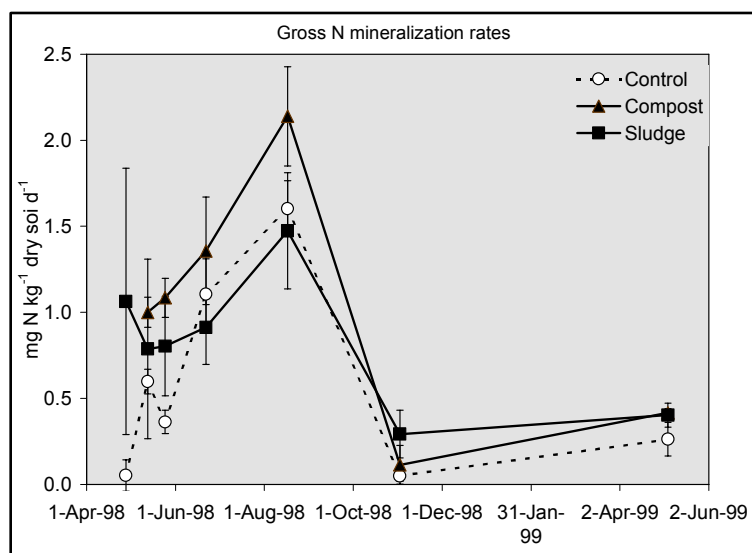


FIG. 1. Gross N mineralization in field microplots treated with domestic sewage sludge and composted waste (bars =  $\pm se$ ).

## REFERENCES

- [1] KIRKHAM, D., BARTHOLOMEW, W.V., Equations for following nutrient transformations in soil utilizing tracer data, *Soil Sci. Soc. Am. Proc.* **18** (1954) 33–34.
- [2] RECOUS, S., et al., In situ changes in gross N transformations in bare soil after addition of straw, *Soil Biol. Biochem.* **31** (1999) 119–133.

# MICROBIAL ASSIMILATION OF ORGANIC N FROM DECOMPOSING CROP RESIDUES DETERMINED USING $^{15}\text{N}$ POOL DILUTION AND MIRROR IMAGING

E.S. JENSEN

Department of Agricultural Sciences, The Agricultural University, Tåstrup, Denmark  
Plant Biology and Biogeochemistry Department, Risø National Laboratory, Roskilde, Denmark

## Abstract

During initial decomposition of crop residues, soluble organic N compounds may either be assimilated directly by the microbial biomass or assimilated as  $\text{NH}_4$  (or  $\text{NO}_3$ ) after exocellular enzymatic breakdown of organic N compounds to  $\text{NH}_4$ . This study aimed at determining the relative role of these pathways of residue N immobilization during initial decomposition (<2 days) of pea (*Pisum sativum* L.) straw (C:N 23:1) in a sandy loam soil using  $^{15}\text{N}$  pool dilution and cross-labelling techniques. The results indicated that during the initial 2 days of pea-straw decomposition, a major part (c. 90%) of the residue N assimilated by the soil microbial biomass was assimilated directly as organic N, assuming that the  $\text{NO}_3$ -N immobilization was negligible.

## 1. INTRODUCTION

Following incorporation of crop residues, large amounts of residue N may be found in the soil microbial biomass within hours to days, e.g. c. 20% of barley or pea straw N after 7 days [1]. There is evidence that the microbial biomass assimilates soluble low-molecular-weight organic substances, e.g. amino acids, (by the direct route, d, Fig. 1) [2], and that surplus N is subsequently released from the cells into the soil  $\text{NH}_4$ -pool (r, Fig. 1). A second route of immobilization of residue N is via the inorganic N pool (i, Fig. 1), following deamination of soluble organic substances by exocellular enzymes (s, Fig. 1); this route is known as the mineralization-immobilization turnover (MIT) pathway [3,4].

By using  $^{15}\text{N}$  tracing and modelling, Hadas et al. [5] suggested that both routes operate concurrently, with the direct route dominating the substrate-specific population and the MIT route operating at the level of the native population. This has been confirmed by Barraclough [6] using  $^{15}\text{N}$ -labelled amino acids and mirror imaging; he found that the direct pathway dominated during the initial decomposition of leucine and glycine. In order to improve models of N mineralization-immobilization in soil, more knowledge is required of the role of each route for the improved interpretation of N-cycle processes during decomposition of organic substances.

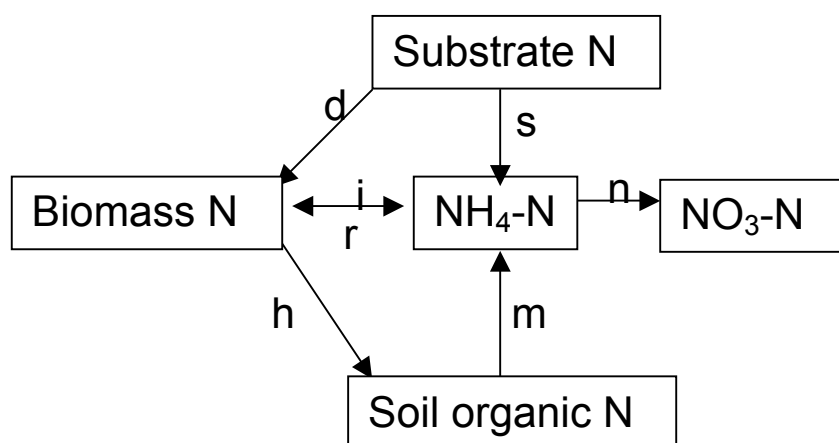


FIG. 1. Simplified schematic presentation of processes and N-pools during the initial decomposition of a substrate (e.g. crop residue), m: mineralization of soil organic N, s: mineralization of substrate N by exocellular enzymes, i: immobilization of ammonium N, r: release of ammonium from microbial cells, d: direct assimilation of residue organic N, n: nitrification, and h: humification of microbial biomass N. The remineralization of labelled N and the immobilization of nitrate were considered to be insignificant during the 0 to 2 days of incubation. Modified after [8].

Mirror imaging or cross-labelling [7] and pool-dilution  $^{15}\text{N}$  techniques [6] can be used to determine process rates of the soil N cycle. The principle of mirror imaging is to label different pools in different treatments, e.g. labelled crop residues and non-labelled ammonium in one treatment and vice-versa. The degree of  $\text{NH}_4$  pool dilution in treatments with and without non-labelled residues will be an indication of the magnitude of the MIT pathway. Using mirror imaging, the proportion of labelled N in the soil microbial biomass derived from labelled residues is a direct measure of the immobilization of residue N. The  $^{15}\text{N}$  enrichment of the ammonium pool will indicate the proportion of ammonium derived from residues. In the treatment with non-labelled residues and labelled ammonium, the immobilization from labelled  $\text{NH}_4$  and the total immobilization from the ammonium pool can be estimated. Knowing the proportion of residue-derived  $\text{NH}_4$  in the labelled residue treatment, it is possible to calculate the amount of labelled residue N immobilized via the ammonium pool. Knowing the total amount of residue N immobilized from the labelled residue treatment, it is possible to obtain an estimate of how much N is immobilized in organic form by difference.

The aim of the study was to determine the relative roles of the MIT and direct routes in the immobilization of residue N during initial decomposition of field pea (*Pisum sativum* L.) straw in a sandy loam soil. The  $^{15}\text{N}$  pool dilution technique was used in combination with  $^{15}\text{N}$  labelled and non-labelled crop residues (mirror imaging) [7].

## 2. MATERIALS AND METHODS

The soil, a sandy loam with 15% clay, was pre-incubated for 1 week. Field-pea residues (straw) were produced in pots with and without  $^{15}\text{N}$  labelling [9] and milled (<1 mm). Residues were either at natural abundance or with approximately 3 atom %  $^{15}\text{N}$  excess. The total-N concentration was 1.8% of DM (C:N 23). Soluble N and C were 0.65 and 7.97%, respectively. There were three treatments:

- C:  $(^{15}\text{NH}_4)_2\text{SO}_4$  – 10.2 atom %  $^{15}\text{N}$  excess –  $10\ \mu\text{g}\ \text{NH}_4\text{-N}\ \text{g}^{-1}$  dry soil;
- L:  $(\text{NH}_4)_2\text{SO}_4$  –  $10\ \mu\text{g}\ \text{NH}_4\text{-N}\ \text{g}^{-1}$  dry soil, with  $^{15}\text{N}$  labelled residues –  $5\ \text{g DM kg}^{-1}$  dry soil;
- U:  $(^{15}\text{NH}_4)_2\text{SO}_4$  – 10.2 atom %  $^{15}\text{N}$  excess –  $10\ \mu\text{g}\ \text{NH}_4\text{-N}\ \text{g}^{-1}$  dry soil, with non-labelled residues –  $5\ \text{g DM kg}^{-1}$  dry soil.

Soil samples, with and without residues were incubated at 55% WHC at 20°C in the dark, and four replicates were used per sampling. Soil was sampled for analysis after 0, 2, 4, and 7 days of incubation. Soil was extracted with KCl for determinations of  $\text{NH}_4$  and  $\text{NO}_3$ , soil microbial biomass was determined by fumigation-extraction ( $k_N$ : 2.22) according to Ref. [10], and soil organic N after three washings with KCl. The  $^{15}\text{N}$  enrichment of each pool was determined by continuous-flow isotope ratio mass spectrometry using elemental analysis coupled online to an isotope ratio mass spectrometer (Finnigan, Delta E).

## 3. RESULTS AND DISCUSSION

The ammonium pool was quickly depleted both with and without residue amendments, due to immobilization and nitrification (Fig. 2A). The nitrate pool increased during the initial 2 days of incubation, due mainly to nitrification, followed by a decline in the residue-amended soils, due to N immobilization (Fig. 2A).

The ammonium pool  $^{15}\text{N}$ -enrichment in the unamended control (C) and in the non-labelled residue treated (U) soil was quickly diluted, due to mineralization of soil and unlabelled residue N concurrently with immobilization and nitrification (Fig. 2B). The  $^{15}\text{N}$  enrichment of the ammonium pool in the two treatments did not change significantly over 2 days, indicating that the MIT route of immobilization played a minor role. However, the  $^{15}\text{N}$  enrichment of the nitrate pool in the residue treatment (U) was lower than that of the unamended control treatment, which indicated that relatively more non-labelled  $\text{NH}_4$  was nitrified in the residue treatment than in the control (Fig. 3C). Averaging the C and U treatments, the total recovery of labelled N was approximately 105% at day 0. After 2 days of incubation, the recoveries were 94% and 87% in the C and U treatments, respectively (data not shown), indicating that gaseous losses of labelled N had occurred in the U treatment.



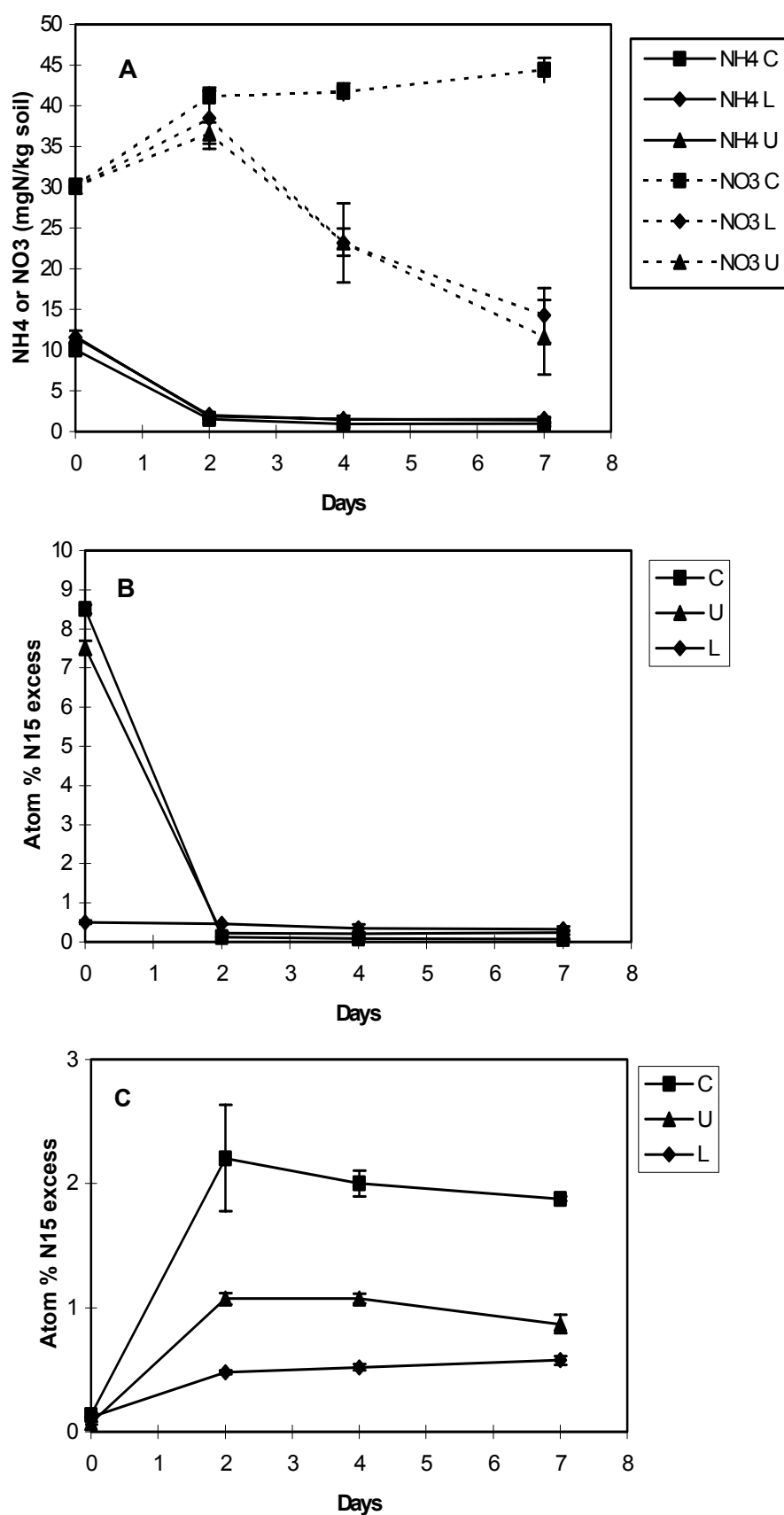


FIG. 2. Inorganic N concentrations and <sup>15</sup>N enrichments during 7 days of incubation. A: NH<sub>4</sub> and NO<sub>3</sub> concentrations, B: NH<sub>4</sub> pool <sup>15</sup>N enrichment, C: NO<sub>3</sub> pool <sup>15</sup>N enrichments. (Bars represent standard deviations).

Data from the initial 2 days of incubation were used to calculate the contributions from the direct and the MIT routes to the immobilization of residue-derived N using the data for the soil microbial biomass. However, labelled N immobilized in the soil microbial biomass, estimated using the standard  $K_N$  of 2.2, was compared with the direct measurement of labelled N immobilized in soil organic N after removal of inorganic N by KCl washings (Fig. 3). The difference between the two methods was not significant, indicating that using the soil microbial biomass N is valid for the measurement of immobilized N.

After 2 days, the soil microbial biomass contained 3.5 mg labelled N  $\text{kg}^{-1}$  soil in treatment U (Fig. 3). Assuming that N was not immobilized from the  $\text{NO}_3$ -pool during the 0 to 2 days of decomposition and no remineralization of labelled immobilized N occurred, it was found that labelled  $\text{NH}_4$  constituted, on average, 38% of the ammonium pool in treatment U. Thus, the total immobilization of  $\text{NH}_4$ -N (labelled and non-labelled) during the 2 days was calculated at 9.2 mg  $\text{NH}_4$ -N  $\text{kg}^{-1}$  soil.

In the treatment with labelled residues (L) and non-labelled ammonium, the residue-derived  $\text{NH}_4$  constituted, on average, 16% of the  $\text{NH}_4$  pool during the 2 days. Thus, it was estimated that residues contributed to the immobilization of N via the MIT route with 1.5 mg N  $\text{kg}^{-1}$  soil ( $0.162 \times 9.2$ ). Total (MIT + direct) immobilization of residue N (L) in the soil microbial biomass was estimated at 15.1 mg N  $\text{kg}^{-1}$  soil (16% of the residue N added, data not shown), indicating that 90% ( $[15.1 - 1.5] \times 100 / 15.1$ ) of the residue N immobilization occurred via the direct route during the initial 2 days of decomposition.

These results are in agreement with the amino acid experiments [5,6] indicating that the direct assimilation of organic N may be a major pathway of soil-N immobilization, especially when the concentration of soluble N is high. The complete dataset can be used for determining the relative roles of the direct and MIT routes during the subsequent periods, e.g. by using a calculation model such as FLUAZ [8]. It has been suggested that the role of soil-N mineralization in the N supply of plants may be overestimated, at least in some terrestrial ecosystems [11]. The role of organic N in plant N supply should be reconsidered, especially in systems with large inputs of organic N, e.g. in animal and/or green manures, such as in organic farming systems.

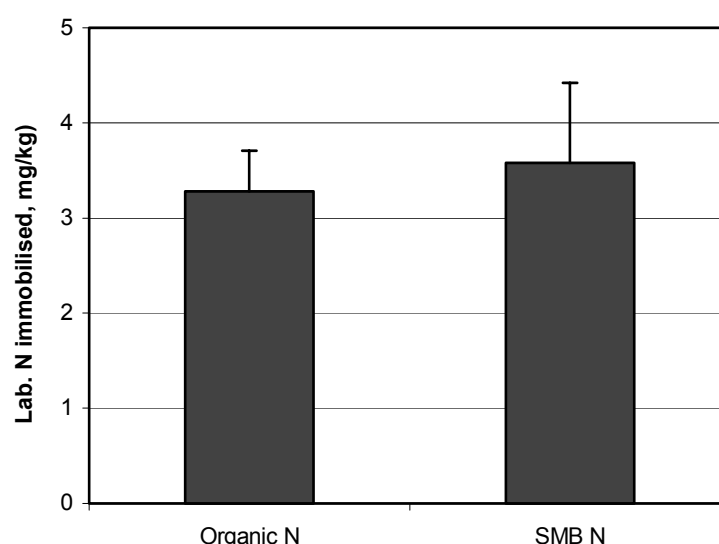


FIG. 3. Comparison of immobilized labelled N in the treatment with non-labelled residues and labelled  $\text{NH}_4$  (U) after 2 days of incubation. Organic N: labelled N immobilized in soil organic N determined after washing with KCl. SMB: labelled N in the soil microbial biomass determined by fumigation-extraction and a  $K_N$  of 2.2 [10]. (Bars represent standard deviations.)

## REFERENCES

- [1] JENSEN, E.S., Nitrogen immobilization and mineralisation during initial decomposition of <sup>15</sup>N labelled pea and barley residues, *Biol. Fertil. Soils* **24** (1996) 39–44.
- [2] BARAK, P., et al., Mineralization of amino acids and evidence of direct assimilation of organic nitrogen, *Soil Sci. Soc. Am. J.* **54** (1990) 769–774.
- [3] JANSSON, S.L., PERSSON, J., “Mineralization and immobilisation of soil nitrogen”, *Nitrogen in Agricultural Soils*, Vol. 22 (STEVENSON, F.J., Ed.), ASA, Madison (1982) 229–252.
- [4] DRURY, C.F., et al., Availability of NH<sub>4</sub> to microorganisms and the soil internal N cycle, *Soil Biol. Biochem.* **23** (1991) 165–169.
- [5] HADAS, A., et al., Assimilation of nitrogen by soil microbial population: NH<sub>4</sub> versus organic N, *Soil Biol. Biochem.* **24** (1992) 137–143.
- [6] BARRACLOUGH, D., The direct or MIT route for nitrogen immobilization: a <sup>15</sup>N mirror image study with leucine and glycine, *Soil Biol. Biochem.* **29** (1997) 101–108.
- [7] WATKINS, N., BARRACLOUGH, D., Gross nitrogen mineralisation during the decomposition of plant residues, *Soil Biol. Biochem.* **28** (1994) 169–175.
- [8] MARY, B., et al., A model for calculating nitrogen fluxes in soil using <sup>15</sup>N tracing, *Soil Biol. Biochem.* **30** (1998) 1963–1979.
- [9] JENSEN, E.S., Dynamics of mature pea residue nitrogen turnover in unplanted soil under field conditions, *Soil Biol. Biochem.* **26** (1994) 455–464.
- [10] BROOKES, P.C., et al., Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil, *Soil Biol. Biochem.* **17** (1985) 837–842.
- [11] NÄSHOLM, T., et al., Boreal forest plants take up organic nitrogen, *Nature* **392** (1998) 914–916.

## EFFECTS OF TILLAGE AND CROPPING ON THE DYNAMICS OF SOIL ORGANIC MATTER

K.M. MANJIAH\*, R.P. VORONEY

Department of Land Resource Science,  
University of Guelph, Guelph, Ontario, Canada

### Abstract

Understanding the dynamics of soil C is the key to maintaining soil organic matter to enhance soil quality and ecosystem functioning, and reduce trace gas emissions from soils. Soils from three sites of eastern Canada (Delhi, Elora, and Harrow) representing different soil textures and management systems were used for the present study. The incubation experiment with  $^{14}\text{C}$ -labelled corn residue decomposition (for 220 days), carried out in controlled laboratory conditions, provided information on the rate of decomposition of labelled residue C and stabilization of C in active microbial fractions. It was observed that the forest soils significantly lowered residue-C mineralization. The residue-C mineralization in soils used for corn grown under conventional and no-till management were less influenced by soil texture. Carbon-13 natural abundance was used to study the dynamics of soil organic matter in long term field experiments. Measurements of  $\delta^{13}\text{C}$  in surface and subsurface soil samples collected from the above mentioned tillage and crop management systems provided information on incorporation of plant-residue C into various components of soil organic matter, soil microbial biomass, water-soluble organic C, and light and humic fractions. Reduced tillage, adopted for 11 to 15 years, did not help to restore soil C levels. Furthermore, the amount of corn-derived C ( $\text{C}_4\text{-C}$ ) incorporated into soil organic matter was not altered by tillage practice. This study revealed that a high proportion of the substrate available to soil microbial biomass during the season is derived from  $\text{C}_3\text{-C}$  in soil. No tillage decreased the light fraction C in the soil or the total quantity of  $\text{C}_4\text{-C}$  in the light fraction. The humin fraction was the most depleted in  $^{13}\text{C}$  whereas fulvic acid was the most enriched fraction. The data generated through these studies will be useful for quantifying progressive incorporation of plant-residue C into organic matter fractions for better understanding of C-transformation and stabilization in soil.

### 1. INTRODUCTION

A better understanding of how organic matter affects soil quality, ecosystem functioning, and atmospheric  $\text{CO}_2$  concentrations would be gained through knowledge of its dynamics. The amount of organic matter in soil is a function of the plant residues that are returned to it and the rate at which those residues decompose. Soil organic matter (SOM) comprises diverse constituents that vary in mass and rate of turnover. Its dynamics reflect the biological activity, soil properties, and the quantity and quality of plant residues returned to the soil. Humus is the largest, most-stable pool of C, comprising mostly resistant material. The soil microbial biomass is a source of, and sink for, biologically mediated nutrients, and is responsible for transforming organic matter and nutrients. Water-soluble organic C (WSOC) is not only a C source for microorganisms, but its production is also believed to be microbially mediated [1]. The light fraction (LF) is a transitory pool of organic matter between fresh plant residues and humus. The LF concentration is highly variable and depends on the amount and characteristics of C inputs and soil-environment factors that affect rates of decomposition [2]. The LF has been suggested as a sensitive indicator of changes in SOM because of its responsiveness to management practices [3]. Field studies have supported this concept, and have shown that SOM accumulation is linked to accumulation of LF [4,5]. Measurement of small and more-active (labile) fractions have, however, been used successfully to identify early changes in SOM as influenced by changes in soil-management practices [6]. These labile fractions are important components of SOM quality, which influences crop productivity.

Carbon-14-labelled substrates have been used, and plant materials incorporated into soils, to investigate decomposition processes and formation of SOM [7–11]. Measuring rates of evolution of  $^{14}\text{CO}_2$  has

---

\*Present address: Nuclear Research Laboratory, Indian Agricultural Research Institute, New Delhi, India.

followed decomposition or rates of decrease in the amounts of added  $^{14}\text{C}$  substance to study short term kinetics of SOM. It has been shown that  $^{13}\text{C}$  is useful for elucidating long term organic matter dynamics in agricultural soils. The  $^{13}\text{C}$  content of SOM corresponds closely to the  $^{13}\text{C}$  content of plant material from which it is derived. Natural variations in  $^{13}\text{C}$ : $^{12}\text{C}$  ratio have been used to assess the fate of recent organic inputs. Terrestrial plants with different photosynthetic pathways can introduce variations of  $^{13}\text{C}$ : $^{12}\text{C}$  in organic fractions. By studying crop-rotation interactions, soil organic carbon (SOC) turnover rates and soil organic fractions remaining in soils can be characterized. Differences in natural abundance of  $^{13}\text{C}$  between C3 and C4 plants have been used as in situ labelling of organic matter for determination of organic C turnover [12]. The  $\delta^{13}\text{C}$  values of the SOC can be used to differentiate the nature of plant materials contributing to the SOM.

Most of the arable soils in Ontario were first cultivated within the last 150 years. The conversion of native forests to arable agriculture resulted in losses of SOC of about 25 to 35% [13]. Janzen et al. [14] inferred from their studies that the decline in soil C resulting from the conversion of soils to arable agriculture has abated. They suggested that the current changes observed in soil C dynamics are a function of current management practices and the effects of these practices usually overshadow any lingering effects of the initial cultivation. The present investigation was undertaken to study the short and long term effects of continuous corn cultivation and tillage on the turnover of SOM and fractions of SOM, and on the rate and extent of organic matter decomposition in contrasting textured soils in Ontario, Canada, using  $^{14}\text{C}$  and  $^{13}\text{C}$ .

## 2. MATERIALS AND METHODS

### 2.1. $^{13}\text{C}$ studies

Soil samples, collected during autumn 1999, from two long term field experiments involving continuous corn managed under conventional and no-tillage (11–15 yr) located at Delhi (a Fox loamy sand) and Harrow (a Brookston clay loam) in eastern Canada, were used for the present study. Winter rye-tobacco and brome grass plots were used as background samples from Delhi and Harrow, respectively. Soil samples were ball-milled and analysed for total C and stable C isotope ratios using a tracemass isotope ratio mass spectrometer (Europa Scientific, Crew, UK) interfaced to a Roboprep unit [15]. Tests with HCl were used to assure no inorganic C was in the soil samples. The natural abundance of heavy isotopes is expressed as parts per thousand (‰), relative to the international Pee Dee Belemnite (PDB) standard using delta-units ( $\delta$ ). The  $\delta^{13}\text{C}$  value was calculated from the measured C isotope ratios of the sample and standard gases as:

$$\delta^{13}\text{C} (\text{‰}) = \frac{(R_{\text{sample}} - R_{\text{standard}})}{R_{\text{standard}}} \times 1000$$

where

R is the  $^{13}\text{C}$ : $^{12}\text{C}$  ratio of the sample or standard gas.

The proportion (X) of soil C derived from corn residues since the experiment was initiated was calculated as:

$$X = \frac{(\delta_s - \delta_{\text{ref}})}{(\delta_{\text{cr}} - \delta_{\text{ref}})} \times 1000$$

where

$\delta_s$  is the  $\delta^{13}\text{C}$  value of sample from corn soil,

$\delta_{\text{cr}}$  is  $-12.1\text{‰}$ , the mean  $\delta^{13}\text{C}$  value of corn residues measured on four replicates of each of leaves, stalks and roots,

and  $\delta_{\text{ref}}$  is  $\delta^{13}\text{C}$  value of the reference soil (brome grass plots in Harrow and tobacco-rye plots in Delhi).

Water-soluble organic carbon was extracted by boiling the soil samples (20 g) in ultrapure water (100 mL) for 60 min. Soluble C in the ultrapure water extracts was obtained by centrifugation at 10,000 rpm for 10 min and filtering through a 0.45- $\mu$ m fibreglass filter using a suction funnel. Soluble organic C was determined on an autoanalyser. After determining soluble C in the ultrapure water extract, the solution samples containing small amounts of inorganic C were acidified to pH 3 with 50 mM H<sub>2</sub>SO<sub>4</sub> and then freeze-dried. The stable C-isotope ratios were also measured using the tracemass isotope ratio mass spectrometer interfaced to a Roboprep unit. The stable C isotope ratios and the proportion of C derived from corn residues since the experiment was initiated were calculated as explained above.

Soil microbial biomass carbon (MBC) was determined by the chloroform-fumigation extraction method [16]. Moist field soils (2-mm sieved) were extracted with 0.5 M K<sub>2</sub>SO<sub>4</sub> with or without fumigation. For <sup>13</sup>C analysis, fumigated and non-fumigated extracts were dialysed in a multiple dialyser to remove sulphate (from K<sub>2</sub>SO<sub>4</sub>) contained in the sample. The samples were freeze-dried and analysed for stable C isotope ratio using the tracemass isotope ratio mass spectrometer. The stable C isotope ( $\delta^{13}\text{C}$ ) value was calculated from the measured C-isotope ratios of the sample and the standard gases as explained in the above section. The  $\delta^{13}\text{C}$  of MBC was estimated as the  $\delta^{13}\text{C}$  of the C extracted from the fumigated sample in excess of that extracted from the non-fumigated sample, as follows:

$$\delta^{13}\text{C SMB} = \frac{(\delta^{13}\text{C}_{\text{FUM}} \times C_{\text{FUM}} - \delta^{13}\text{C}_{\text{CNT}} \times C_{\text{CNT}})}{(C_{\text{FUM}} - C_{\text{CNT}})}$$

where

$C_{\text{FUM}}$  is the amount of C extracted from fumigated samples (mg C kg<sup>-1</sup>),

$C_{\text{CNT}}$  is the amount of C extracted from non-fumigated samples (mg C kg<sup>-1</sup>),

$\delta^{13}\text{C}_{\text{FUM}}$  is <sup>13</sup>C natural abundance for fumigated extracts (‰),

$\delta^{13}\text{C}_{\text{CNT}}$  is <sup>13</sup>C natural abundance for non-fumigated extracts (‰).

After the extraction of WSOC, the soil samples were used for light fraction (LF) organic matter separation. The LF organic matter in soil samples was separated by flotation on a solution of NaI with the density adjusted to 1.7 g cm<sup>-3</sup>, dried at 60°C, and ground to pass through a 125- $\mu$ m sieve. The heavy fraction (HF) resulting from the separation of LF was retained for extraction of humic substances. A fraction of the LF (125  $\mu$ m) samples was analysed for stable C ( $\delta^{13}\text{C}$ ) and the fraction of C originating from corn (fC4) was calculated as explained above.

The humic substances were extracted from the HF obtained by the removal of WSOC and LF organic matter fraction. For the extraction of humus (0.1 M Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>·10H<sub>2</sub>O) and fractionation of humic substances (humic and fulvic acids), the procedure of Schnitzer and Schuppli [17] was followed. The humus was further fractionated according to molecular size, using Spectra/Por membranes, in a multiple dialyser. This molecular separation was done to isolate humic substances, which have molecular weights larger than 10,000, 50,000 and 100,000, analysed for stable C ( $\delta^{13}\text{C}$ ) and the fraction of C originating from corn in different humic fractions (fC4) was calculated from a two end-member mixing model as described earlier.

## 2.2. <sup>14</sup>C studies

Intact soil cores from the surface soil layer (0–5 cm) of a conventionally tilled and a no-till site cropped to continuous corn, located at the Delhi, Elora, and Harrow Research Stations, were used. The three soils represent a range of textures found in southern Ontario, i.e., (i) a Fox loamy sand, (ii) a Wellington silt loam, and (iii) a Brookston clay loam, respectively. Carbon-14-labelled corn residues were added to each of the soils in such a way as to approximate previous residue management, i.e. crop residues applied to the conventionally tilled treatment were thoroughly mixed with the soil whereas those applied to the no-till soils were applied to the soil surface. To serve as controls, soil cores were obtained from adjacent sites that had never been cropped to corn and from forested sites. Soils were incubated (by adding <sup>14</sup>C-labelled corn leaves at 1 mg C g<sup>-1</sup> soil, with a specific activity of 222.26

Bq/mg C) and sub-sampled periodically to determine the total C and residue-derived  $^{14}\text{C}$  associated with the microbial biomass.

### 3. RESULTS AND DISCUSSION

#### 3.1. Total soil organic carbon

Tillage method under corn did not affect the amount of organic C in the soils studied (Table I). The NT had more organic C in the surface layer (0–10 cm) than did the CT. In the deeper soil layers, the organic C content was slightly higher in CT than in NT. The organic C content in tobacco-rye plots was lower than in the corn plots under different tillage treatments at the Delhi site. But at Harrow, the bromegrass plots had higher organic C as compared to the corn plots. The tobacco-rye and bromegrass plots, which were used as the reference/background soils in Delhi and Harrow, had  $\delta^{13}\text{C}$  values typical of C3 vegetation, ranging from  $-25.2$  to  $-26.2$  (Table I). The  $\delta^{13}\text{C}$  values of SOM from corn under CT were more enriched at all soil depths compared to corn under NT plots. However, under the NT plots, more C enrichment was observed at 0 to 10 cm at both the sites. In general, the results show that tillage had no significant effect on  $\delta^{13}\text{C}$  of soil. The SOM under continuous corn growing with either of the tillage practices was significantly enriched in  $\delta^{13}\text{C}$  in comparison with the reference background soils at both of the sites.

TABLE I. TOTAL SOIL ORGANIC CARBON (TSOC),  $\delta^{13}\text{C}$  OF SOIL ORGANIC MATTER, CORN-DERIVED CARBON IN SOIL ORGANIC MATTER AND TURNOVER OF NATIVE SOIL ORGANIC MATTER (C3-C) IN DELHI AND HARROW SOILS AS AFFECTED BY CORN CROPPING AND TILLAGE

Treatment	Depth (cm)	Total C (Mg C ha <sup>-1</sup> )	$\delta^{13}\text{C}$ (‰)	C4-C (%)	C3-C (Mg C ha <sup>-1</sup> )	k ( $\times 10^{-3}$ )	T <sub>1/2</sub> (yr)
<b>Delhi</b>							
TB-Rye	0–10	5.2	$-26.2$				
	10–20	4.9	$-26.2$				
	20–30	3.1	$-26.0$				
CC-NT	0–10	9.9	$-24.0$	15.8	8.3	0.042	17
	10–20	9.9	$-24.6$	10.9	7.0	0.032	22
	20–30	3.0	$-25.1$	6.70	2.8	0.009	75
CC-CT	0–10	9.0	$-23.8$	17.4	7.4	0.032	22
	10–20	7.3	$-24.2$	13.8	6.3	0.023	30
	20–30	4.0	$-24.8$	8.80	3.6	0.014	51
<b>Harrow</b>							
BG	0–10	30.2	$-25.4$				
	10–20	27.3	$-25.2$				
	20–30	16.7	$-25.2$				
CC-NT	0–10	22.2	$-22.3$	23.3	17.0	0.038	18
	10–20	18.1	$-23.5$	13.5	15.7	0.037	19
	20–30	12.9	$-24.2$	8.30	11.8	0.023	30
CC-CT	0–10	21.0	$-22.1$	24.8	15.8	0.043	16
	10–20	19.5	$-23.2$	15.7	16.4	0.034	20
	20–30	11.5	$-23.6$	12.4	10.1	0.034	20

At both the sites, NT resulted in a significantly higher proportion of C4-C in SOM in the surface layer (0-10 cm) compared with CT; however, at deeper soil depths, corn under CT had significantly higher proportions of C4-C in SOM compared with corn under NT. As compared to the coarse-textured Delhi soils, the proportion of C4-C in SOM and also the total quantity of C4-C were significantly higher in fine textured Harrow soils for all soil depths. The quantity of C4-C in soil, when calculated as a total ( $\text{Mg C ha}^{-1}$ ), in the 0- to 30-cm depth, was not affected by tillage at either site and the corn under CT had 15.5% of its SOM derived from C4-C, compared with 13.1% under NT. Reduced tillage under corn did not cause any significant change in SOM compared with conventional tillage practices in these two soils. No-tillage plots had more C in the 0- to 10-cm layer, probably due to less incorporation compared to CT. These results are consistent with those from previous studies on the effects of tillage on SOM levels [18,19].

The 0- to 20-cm layer of the CT soil at Delhi had 15.6% of its C derived from corn in comparison with 13.4% in the NT soil, after 11 years of continuous corn cultivation. At Harrow, the 0- to 20-cm layer of CT had 20.3% and 18.4% in NT treatment after 15 years of continuous corn. Wanniarachchi et al. [19] reported 12% of C4-C with CT and 9% C4-C with NT in Delhi soils (0–20 cm) after 6 years of cropping, and 26% C4-C in Elora soils after 29 years of continuous cropping. Balesdent et al. [20] compared three tillage practices, conventional (CT), superficial (ST), and no tillage (NT) for storage of C4-C in soil, and observed that, after 17 year of continuous corn, NT had the lowest (20%) amount of C4-C in soil compared with 30% in both CT and ST for a soil depth of 0 to 30 cm. The percentage of C4-C in the 0- to 20-cm soil layer for both CT and NT after 15 years of continuous corn cropping at Harrow is in agreement with previous studies in Ontario. Gregorich et al. [21] found that, after 25 years of continuous corn cropping, the 0- to 27-cm layer had 28% of the total C as C4-C. In another study, Gregorich et al. [22] reported that, after 32 years of continuous corn under fertilized conditions, the 0- to 26-cm layer had 25% as C4-C; however, under unfertilized conditions, this figure was 16.5% indicating the importance of quantity of corn-residue input on the proportion of C4-C in soil. In a study in eastern Quebec, Angers et al. [18] observed that, after 11 years of silage corn under three tillage methods (conventional, ridge, and surface), 6 to 7% of C4-C was sequestered in the 0- to 24-cm layer.

The amounts of C3-C in soil were not significantly affected by tillage at either site. Assuming that the C levels in tobacco-rye plots at Delhi and brome grass at Harrow were not significantly changed during the study, it is possible to calculate the decay rate ( $k$ ) of C3-C under corn using a first-order equation ( $A_t = A_0 e^{-kt}$ ). The  $k$  values for the SOM in different soil layers for corn under CT and NT did not differ significantly; the half-life of C3-C under CT in the 0- to 20-cm depth was 18 to 26 years compared with 18 to 20 years under NT. It appears that, in the short term (11 to 15 years), tillage method does not show any significant effect on the decomposition of C3-C. Gregorich et al. [21] estimated a half-life of 13 years for the C3-C in the 0- to 15-cm layer of an eastern Ontario soil cropped to continuous corn for 25 years, and a half-life for C3-C in the 0- to 30-cm layer of 24 years. In another study, Gregorich et al. [22] estimated a half-life of 19 years for C3-C in the 0- to 10-cm layer of a southern Ontario soil continuously cropped to corn for 32 years. Wanniarachchi et al. [19] observed half-lives of 24 and 27 years in coarse-textured Delhi soil (0–15 cm) for continuous corn for 6 years under CT and NT treatments, respectively.

Conventional tillage plots at Delhi (11 years) sequestered  $1.85 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$  of corn-derived C in the 0- to 30-cm layer compared to  $1.89 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$  under no tillage. At Harrow, sequestration of corn-derived C in the 0- to 30-cm layer of continuous corn under CT and NT (15 years) was 3.47 and 3.55  $\text{Mg C ha}^{-1} \text{ yr}^{-1}$ , respectively. It is evident from these data that reduced-tillage practices have no beneficial effects on the sequestration of corn-derived C in soil. However, the fine-textured soil at Harrow appeared to be more effective in sequestering corn-derived C than did the coarse-textured soil at Delhi, although the corn-residue inputs to soil were somewhat similar at both locations.

### 3.2. Water soluble organic carbon

The amount of water-soluble organic carbon (WSOC) was slightly lower under continuous corn than under reference tobacco-rye and brome grass plots. Tillage treatment did not alter greatly the WSOC



content. The WSOC  $\delta^{13}\text{C}$  values for the corn soils were less negative than those for reference plots (Table II) due to the input of C4-derived C. Most of the C in WSOC was derived from C3 vegetation. The proportion of C4-C ranged from 21.2 to 28.6% and 17.0 to 25.3% under CT and NT, respectively, at Delhi, and from 19.0 to 22.8% under CT and 14.1 to 22.8% under NT at Harrow. In the Delhi soil, the slightly higher contribution of C4-C to the WSOC can be attributed to the coarse texture. The  $\delta^{13}\text{C}$  values of the WSOC were strongly correlated with those for the whole soil ( $r^2=0.78$ ,  $P=0.005$ ), suggesting that the isotopic composition of WSOC was in equilibrium with that of the whole soil C.

### 3.3. Microbial biomass carbon

Eleven years of corn cultivation under NT in a coarse-textured soil resulted in nearly 20% more soil microbial biomass C (SMBC) in the soil surface (0–10 cm) compared to CT, whereas it was 25% more in fine textured soil after 15 years of continuous corn cultivation; these results support the findings of others in similar studies. When the whole 0- to 30-cm layer of Delhi soil is considered, NT and CT had similar SMBC levels. This supported previous findings of large increases in SMBC under NT at the soil surface, but when the entire depth of tillage was considered, only small differences or reductions resulted.

TABLE II.  $\delta^{13}\text{C}$  AND CORN-DERIVED CARBON (C4-C) IN WATER SOLUBLE ORGANIC CARBON (WSOC) AND MICROBIAL BIOMASS CARBON (MBC) IN DELHI AND HARROW SOILS AS INFLUENCED BY CORN CROPPING AND TILLAGE

Treatment Depth (cm)		WSOC			MBC		
		WSOC (Mg C ha <sup>-1</sup> )	δ <sup>13</sup> C (‰)	C4-C (%)	MBC (Mg C ha <sup>-1</sup> )	δ <sup>13</sup> C (‰)	C4-C (%)
Delhi							
TB-R	0–10	0.15	–26.1		0.35	–24.6	
	10–20	0.19	–26.2		0.17	–25.2	
	20–30	0.13	–26.2		0.10	–24.3	
CC-NT	0–10	0.32	–22.6	25.3	0.44	–16.4	65.9
	10–20	0.21	–23.4	20.1	0.20	–18.7	49.7
	20–30	0.11	–23.8	17.0	0.08	–19.4	40.3
CC-CT	0–10	0.29	–22.1	28.6	0.32	–15.7	71.4
	10–20	0.22	–22.3	27.6	0.19	–18.0	55.2
	20–30	0.15	–23.2	21.2	0.11	–17.0	59.7
Harrow							
BG	0–10	0.72	–25.0		0.66	–24.5	
	10–20	0.52	–25.1		0.40	–24.5	
	20–30	0.35	–25.2		0.29	–25.0	
CC-NT	0–10	0.52	–22.1	22.8	0.57	–17.2	58.6
	10–20	0.39	–23.0	16.1	0.34	–18.8	46.0
	20–30	0.31	–23.3	14.1	0.29	–20.8	32.4
CC-CT	0–10	0.62	–22.1	22.8	0.46	–16.5	64.9
	10–20	0.41	–22.6	19.2	0.36	–18.2	51.0
	20–30	0.33	–22.7	19.0	0.29	–19.9	39.6

Franzluebbers et al. [23] found 73 to 146% higher SMBC concentration under NT than CT for the 0 to 5 cm layer, but only 12 to 43% more for the 0 to 20 cm depth in different wheat cropping systems. Granatstein et al. [24] found that the effect of tillage practice on SMBC was most pronounced in the surface 5-cm layer. These differences between NT and CT can be attributed largely to the high level of crop residues accumulated on the soil surface under NT.

Proportions of SMBC to total organic C (TOC) were used to assess tillage and cropping effects on the quantity of SOM present as SMB. Microbial biomass accounts for 1 to 5% of the TOC in soil [25]. The SMB responds more quickly to changes in management than does the TOC, which is relatively slow to change. Therefore, SMBC/TOC has been suggested as a sensitive indicator of change in SOM [26]. The SMBC/TOC increased under NT compared to CT in the 0- to 10-cm layers of both Delhi and Harrow soils cropped to corn. Franzluebbers et al. [23] observed similar results under NT compared to CT. Powlson et al. [26] observed an increase in the SMBC/TOC with incorporation of straw. Continuous growing of brome grass at Harrow produced a high SMBC/TOC due to higher SMBC levels. Sparling [27] observed that continuous cultivation of corn caused a decline in SMBC/TOC compared with permanent pasture. It can be assumed that SMBC/TOC observed under the different tillage and cropping systems in this study is a reflection of amounts of crop-residue inputs to the soil. High levels of available substrates in soil provide better conditions for the SMB to proliferate compared to substrate-limited soils.

The  $\delta^{13}\text{C}$  values for the microbial biomass were substantially less negative than those of WSOC in soils under continuous corn, reflecting the enrichment of  $^{13}\text{C}$  and the greater contribution of corn-residue C to the microbial biomass. Ryan et al. [28] reported about a two-fold difference between the %C4-C in microbial and extractable C. Across all of the treatments, 32 to 71% of the MBC was derived from corn (Table II). Most of the MBC was derived from corn residue even though more than 75% of soil C originated from C3-plants. The data are consistent with those of Ryan and Aravena [29], who reported that 55% of the MBC was derived from C4-C after 5 years of continuous corn. Angers et al. [18] found that up to 35% of MBC was C4-C derived after 11 years of continuous corn. Ryan and Aravena [29] observed that after 5 years of continuous corn in a coarse-textured soil, SMB of NT and CT had 30% and 50% C4-C, respectively. In the present study, proportions of C4-C in SMB after 11 years of corn were 58% and 63%, for NT and CT, respectively (averaged 0–20 cm). However, the surface of CT soil (0–10 cm) in this study had a higher proportion of C4-C in the SMB compared to NT soil. Corn under NT in Harrow resulted in less C4-C in SMB (53%) compared to corn under CT (58%) at 0 to 20 cm after 15 years of continuous corn cultivation. Angers et al. [18] compared three tillage systems on C4-C in SMB in the soil surface (0–8 cm) and found that surface tillage had the highest (33%) proportion of C4-C in SMB compared to ridge tillage (21%) and conventional tillage (20%) after 11 years of silage corn. In a 1-year study of  $\delta^{13}\text{C}$ , Bruulsema and Duxbury [30] found that the MBC was derived from corn C produced during the growing season.

### 3.4. Light fraction carbon

The light-fraction C (LF-C) was slightly less in the coarse-textured Delhi soil than in the Harrow soil. (Table III). Tillage treatment did not show a significant difference in the LF-C, although CT always had higher LF-C values than did NT. The LFs of the continuous-corn plots were enriched in  $^{13}\text{C}$  (a  $\delta^{13}\text{C}$  value of  $-17.0$  to  $-23.7$ ), which indicated that 60 to 65% of the C was derived from corn in the surface 0- to 10-cm layer. The relatively higher amount of LF-C with CT indicates that this pool of organic matter is susceptible to change in management practice. This partially decomposed organic matter also accounted for 6% and 5% of the C4-C in the surface 10 cm of soil in CT and NT at Delhi and 7% and 6% of the C4-C in the surface 10 cm in CT and NT at Harrow, respectively. The labile nature of the LF is shown by the fact that 50% and 72% of the original C3-C at Delhi and Harrow had turned over since the start of the experiment, as compared to 94% reported by Gregorich et al. [22]. The estimated half-life of LF C3-C in corn plots for 0 to 10 cm at both the sites was 8 to 11 years, the same as estimated by Gregorich et al. [21, 22]. This half-life is similar to those reported in studies using  $^{14}\text{C}$ -labelled plant residues [10].

TABLE III. AMOUNT OF LIGHT FRACTION (LF) ORGANIC MATTER,  $\delta^{13}\text{C}$  OF LF-C, CORN-DERIVED CARBON IN LF-C, DECAY CONSTANT OF NATIVE LF-C (C3-C) AND RATIO OF LF-C TO TOTAL SOIL ORGANIC CARBON IN DELHI AND HARROW SOILS AS INFLUENCED BY CORN CROPPING AND TILLAGE

Treatment	Depth (cm)	LF-C (g kg <sup>-1</sup> )	$\delta^{13}\text{C}$ (‰)	C4-C (%)	C3-C (Mg C ha <sup>-1</sup> )	k (x10 <sup>-3</sup> )	(LF-C/TSOC) (%)
Delhi							
TB-R	0–10	8.76	–27.3				12.0
	10–20	8.8	–27.5				7.3
	20–30	5.0	–27.4				8.0
CC-NT	0–10	12.1	–18.2	59.7	0.3	0.062	8.0
	10–20	11.0	–19.2	54.3	0.2	0.034	6.9
	20–30	7.0	–23.7	24.7	0.1	0.056	6.0
CC-CT	0–10	5.1	–17.9	62.2	0.3	0.068	8.8
	10–20	6.9	–17.9	62.6	0.2	0.036	8.9
	20–30	4.4	–20.6	44.4	0.2	0.034	7.8
Harrow							
BG	0–10	8.2	–26.2				10.0
	10–20	4.4	–25.2				7.7
	20–30	4.0	–25.2				9.8
CC-NT	0–10	7.0	–17.9	59.3	0.9	0.081	9.8
	10–20	4.0	–20.1	39.1	0.8	0.065	7.2
	20–30	4.0	–22.0	24.2	0.7	0.058	7.0
CC-CT	0–10	4.6	–17.0	65.3	0.8	0.089	10.8
	10–20	5.0	–18.5	50.3	0.9	0.054	9.7
	20–30	4.0	–21.6	27.3	0.6	0.062	7.8

Conventional-tillage plots had higher LFC/TOC ratios than did NT plots, indicating the presence of more plant residue in the former. Delhi coarse-textured soil had higher LFC/TOC ratios (6–12%) than did Harrow fine-textured soil (7–11%). Janzen et al. [5] reported that LF accounts for 2 to 18% of the total soil C. It seems that conversion of LF to HF is less efficient in coarse-textured soils compared to medium or heavy textured soils. Christensen [31] reported that LF is quantitatively more important in sandy soils than in clayey soils. Light fraction probably decomposes at slower rates and accumulates at higher levels in sandy soils compared to medium or heavy-textured soils. Light-fraction C decreased with depth, a trend observed also by Janzen et al. [5] and Spycher et al. [32], and probably reflects the distribution of plant and microbial debris within the soil profile.

### 3.5. Humic substances

The humic substances were extracted from the heavy fraction of soil after removal of WSOC and LF organic matter, which minimize the possible contamination of humic fractions with plant residues during the extraction process. The data generated in the present study in general indicate that the insoluble heavy fraction (humins) was observed to be more negative in  $\delta^{13}\text{C}$  as compared to the rest of the humic fractions, suggesting that more stable/recalcitrant organic matter is associated with it. These observations were consistent irrespective of soil and tillage system. The acid-soluble fraction (fulvic acid) was shown to be less negative in  $^{13}\text{C}$  values. The humic substances with different molecular weights (10,000, 50,000, and 100,000) showed slight variation with respect to  $^{13}\text{C}$  values among different treatments. Tillage did not have a significant effect on  $\delta^{13}\text{C}$  of different humic fractions or the proportion of C4-C in them.

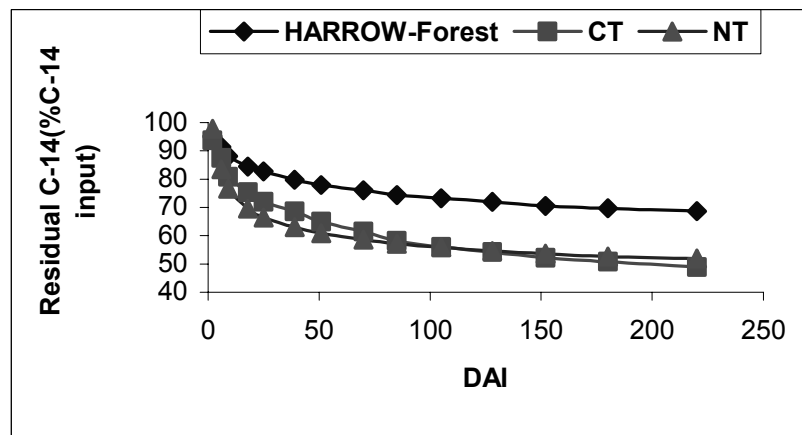
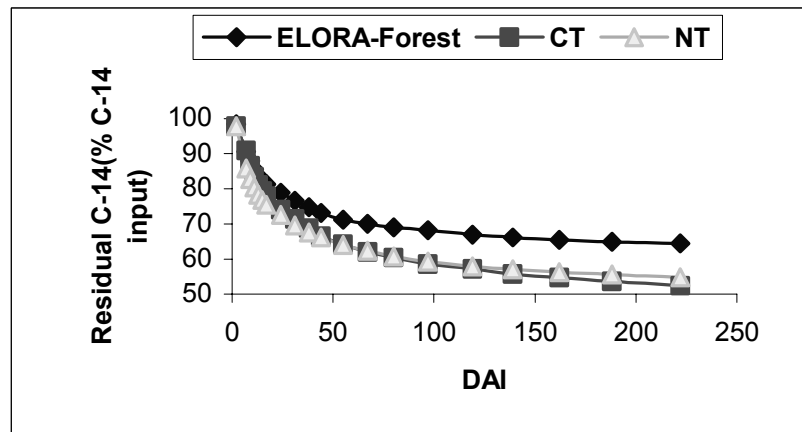
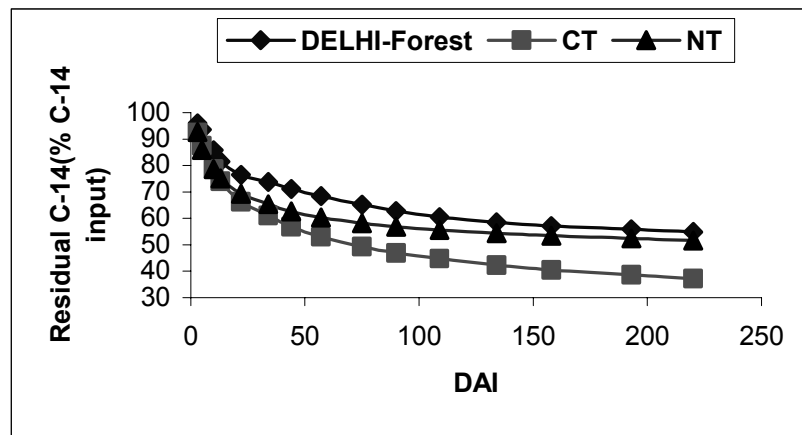


FIG. 1.  $^{14}\text{C}$  remaining in soil (% of  $^{14}\text{C}$  input) at the end of 220 days of incubation in variously textured soils from three locations in Ontario as affected by tillage and corn cropping.

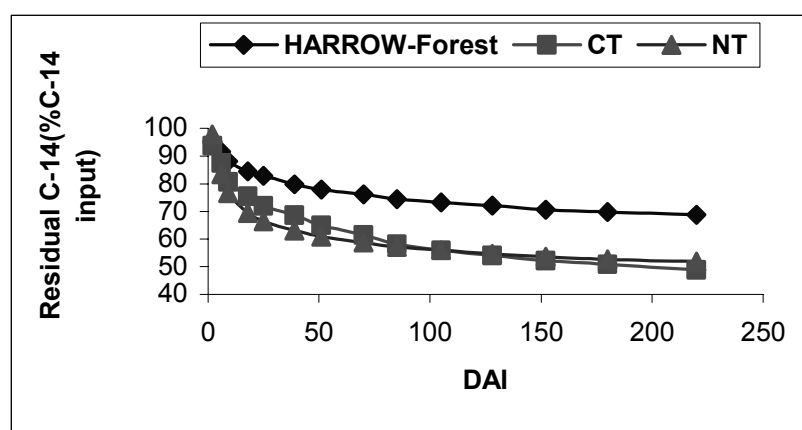
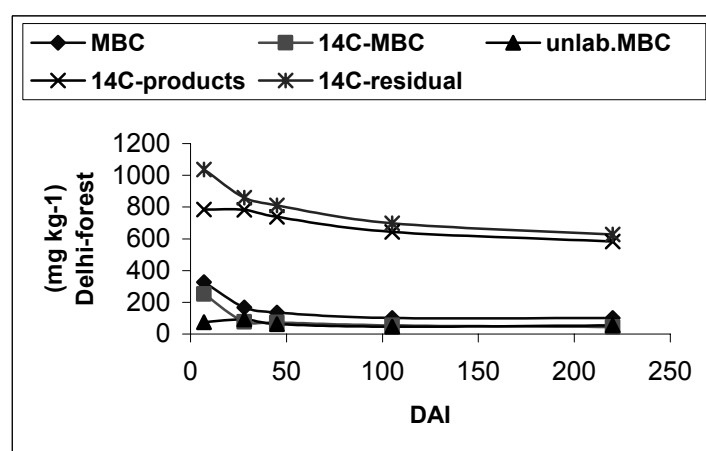
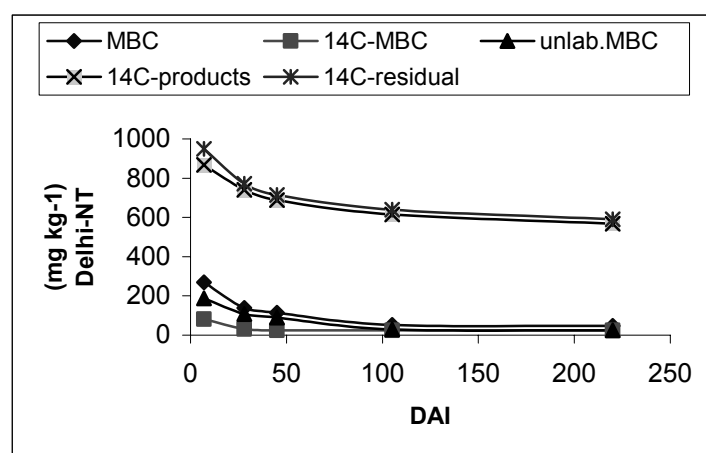


FIG. 2. Total microbial biomass,  $^{14}\text{C}$  microbial biomass, unlabelled microbial biomass,  $^{14}\text{C}$  microbial products and  $^{14}\text{C}$  remaining in soil as affected by tillage and corn cropping in Delhi soils.

### 3.6. $^{14}\text{C}$ -labelled residue decomposition studies

The  $^{14}\text{C}$ -corn residue was rapidly metabolized in the initial 7 days of incubation, irrespective of soil and treatment. After reaching a peak, the rate of  $^{14}\text{CO}_2$  evolution declined rapidly until day 14, after which it declined more slowly. It was observed that, in the initial days of incubation, the residues well mixed with the soils showed higher mineralization than the surface-applied treatments. However, at the end of 220 days of incubation, the proportion of the  $^{14}\text{C}$ -residue C evolved as carbon dioxide was not

significantly affected by tillage treatment, i.e. whether the residue was left on the soil surface or incorporated into the soil. The mineralization of residue C was substantially lower in the forest soils compared to the cropped soils, suggesting that the presence of litter organic matter has an important role in the transformation and stabilization of added crop residues. Soil texture had no significant effect on mineralization of residue C in Elora and Harrow soils (medium to fine texture), however, in Delhi soils (coarse texture) mineralization was substantially higher during this incubation study. At the end of 220 days of incubation, the amount of residue  $^{14}\text{C}$  remaining in soil (% of added  $^{14}\text{C}$  input) followed the sequence forest>NT>CT, i.e. the data ranged from 55 to 69%, 52 to 55% and 37 to 52% in forest, NT and CT treatments, respectively (Fig. 1).

The addition of  $^{14}\text{C}$ -labelled residue at a rate of  $1 \text{ mg C g}^{-1}$  soil resulted in large increases in microbial biomass. Total biomass C increased nearly two-fold within 7 days, but decreased thereafter, and at a relatively more rapidly in the Delhi sandy soil (Fig. 2). The proportion of C derived from the labelled residue and incorporated in the biomass was higher in forest soils (8–22%) as compared to cropped soils (5–8%). On the other hand, the proportion of C derived from the  $^{14}\text{C}$ -labelled residue remaining and incorporated into biomass ranged from 9 to 24% in forest soils and from 6 to 12% in corn-cropped soils. These observations are consistent with the findings of Ladd et al. [9] who reported values of 11 to 15% of input  $^{14}\text{C}$  (21–29% of residual  $^{14}\text{C}$ ). The growth and stabilization of unlabelled biomass C (total biomass C minus labelled biomass C) and  $^{14}\text{C}$ -labelled microbial products (residual  $^{14}\text{C}$ -labelled C minus  $^{14}\text{C}$  microbial biomass C), as influenced by different treatments during the incubation period, are also depicted in Fig. 2. The amount of labelled non-living organic matter, i.e.  $^{14}\text{C}$ -labelled microbial products, which is a readily available energy-rich material, is labile and represents a significant sink for and source of energy and nutrients in the studied soils.

## ACKNOWLEDGEMENTS

The senior author gratefully acknowledges the award of a BOYSCAST fellowship from Department of Science and Technology, Government of India, to carry out the present work with Dr. R.P. Voroney. The  $\delta^{13}\text{C}$  analyses were performed at the Stable Isotope Laboratory, Department of Soil Science, University of Saskatchewan, Canada.

## REFERENCES

- [1] CHRIST, M.J., DAVID, M.B., Temperature and moisture effects on the production of dissolved organic carbon in a spodosol, *Soil Biol. Biochem.* **28** (1996) 1191–1199.
- [2] GREGORICH, E.G., JANZEN, H.H., “Storage of soil carbon in the light fraction and macroorganic matter”, *Structure and Soil Organic Matter Storage in Agricultural Soils* (CARTER, M.R., STEWART, B.A., Eds.), Lewis Publishers, Boca Raton (1995) 167–190.
- [3] GREGORICH, E.G., et al., Towards a minimum data set to assess soil organic matter quality in agricultural soils, *Can. J. Soil Sci.* **74** (1994) 367–385.
- [4] WANDER, M.M., et al., Organic and conventional management effects on biologically active soil organic matter pools, *Soil Sci. Soc. Am. J.* **58** (1994) 1130–1139.
- [5] JANZEN, H.H., et al., Light-fraction organic matter in soils from long term crop rotations, *Soil Sci. Soc. Am. J.* **56** (1992) 1799–1806.
- [6] HAYNES, R.J., Labile organic matter fractions and aggregate stability under short-term, grass-based leys, *Soil Biol. Biochem.* **31** (1999) 1821–1830.
- [7] JENKINSON, D.S., Studies on the decomposition of plant material in soil. IV. The effect of rate of addition, *J. Soil Sci.* **28** (1977) 417–423.
- [8] JENKINSON, D.S., Studies on the decomposition of plant material in soil. V. The effects of plant cover and soil type on the loss of carbon from  $^{14}\text{C}$  labelled ryegrass decomposing under field conditions, *J. Soil Sci.* **28** (1977) 424–434.
- [9] LADD, J.N., et al., Microbial biomass formed from  $^{14}\text{C}$  and  $^{15}\text{N}$ -labelled plant material decomposing in soils in the field, *Soil Biol. Biochem.* **13** (1981) 119–126.
- [10] VORONEY, R.P., et al., Decomposition of wheat straw and stabilization of microbial products, *Can. J. Soil Sci.* **69** (1989) 63–77.

- [11] BOTTLNER, P., et al., Decomposition of  $^{14}\text{C}$  - and  $^{15}\text{N}$  -labelled plant material, under controlled conditions, in coniferous forest soils from a north-south climatic sequence in western Europe, *Soil Biol. Biochem.* **30** (1998) 597–610.
- [12] BALESIDENT, J., et al., Soil organic matter turnover in long term field experiments as revealed by carbon-13 natural abundance, *Soil Sci. Soc. Am. J.* **52** (1988) 118–124.
- [13] ELLERT, B.H., GREGORICH, E.G., Storage of carbon and nitrogen in cultivated and adjacent forested soils of Ontario, *Soil Sci.* **161** (1996) 587–603.
- [14] JANZEN, H.H., et al., “Soil carbon dynamics in Canadian agroecosystems”, *Soil Processes and the Carbon Cycle* (LAL, R., Ed.), CRC Press, Boca Raton (1998) 57–80.
- [15] KNIGHT, J.D., et al., Comparison of a dual-inlet gas isotope ratio mass spectrometry system and an automated single-inlet mass spectrometry system for delta  $^{13}\text{C}$  analysis, *Comm. Soil Sci. Plant Anal.* **25** (1994) 447–454.
- [16] VORONEY, R.P., et al., “Soil microbial biomass C and N”, *Soil Sampling and Methods of Analysis* (CARTER, M.R., Ed.), Lewis Publishers, Boca Raton (1993) 227–286.
- [17] SCHNITZER, M., SCHUPPLI, P., The extraction of organic matter from selected soils and particle size fractions with 0.5 M NaOH and 0.1 M  $\text{Na}_4\text{P}_2\text{O}_7$  solutions, *Can. J. Soil Sci.* **69** (1989) 253–262.
- [18] ANGERS, D.A., et al., Dynamics of soil organic matter and corn residues affected by tillage practices, *Soil Sci. Soc. Am. J.* **59** (1995) 1311–1315.
- [19] WANNIARACHCHI, S.D., et al., Tillage effects on the dynamics of total and corn-residue-derived organic matter in two southern Ontario soils, *Can. J. Soil Sci.* **79** (1999) 473–480.
- [20] BALESIDENT, J., et al., Effect of tillage on soil organic carbon mineralization estimated from  $^{13}\text{C}$  abundance in maize fields, *J. Soil Sci.* **41** (1990) 587–596.
- [21] GREGORICH, E.G., et al., Turnover of soil organic matter and storage of corn residue carbon estimated from natural  $^{13}\text{C}$  abundance, *Can. J. Soil Sci.* **75** (1995) 161–167.
- [22] GREGORICH, E.G., et al., Fertilization effects on soil organic matter turnover and corn residue C storage, *Soil Sci. Soc. Am. J.* **60** (1996) 472–476.
- [23] FRANZLUEBBERS, A.J., et al., Tillage and crop effects on seasonal soil carbon and nitrogen dynamics, *Soil Sci. Soc. Am. J.* **59** (1995) 1618–1624.
- [24] GRANATSTEIN, D.M., et al., Long term tillage and rotation effects on soil microbial biomass, carbon and nitrogen, *Biol. Fertil. Soils* **5** (1987) 265–270.
- [25] SMITH, J.L., PAUL, E.A., “The significance of soil microbial biomass estimations,” *Soil Biochemistry Vol. 6* (BOLLAG, J.M., STOTZKY, G., Ed.), Marcel Dekker, New York (1990) 357–396.
- [26] POWLSON, D.S., et al., Measurement of soil microbial biomass provides an early indication of changes in total soil organic matter due to straw incorporation, *Soil Biol. Biochem.* **19** (1987) 159–164.
- [27] SPARLING, G.P., Ratio of microbial biomass carbon to soil organic carbon as a sensitive indicator of changes in soil organic matter, *Aust. J. Soil Res.* **30** (1992) 195–207.
- [28] RYAN, M.C., et al., “The use of  $^{13}\text{C}$  natural abundance to investigate the turnover of the microbial biomass and active fractions of soil organic matter under two tillage treatments”, *Soils and Global Change* (LAL, R., et al., Ed.), CRC Lewis Publishers, Boca Raton (1995) 351–360.
- [29] RYAN, M.C., ARAVENA, R., Combining  $^{13}\text{C}$  natural abundance and fumigation methods to investigate soil microbial biomass turnover, *Soil Biol. Biochem.* **26** (1994) 1583–1585.
- [30] BRUULSEMA, T.W., DUXBURY, J.M., Simultaneous measurement of soil microbial nitrogen, carbon, and carbon isotope ratio, *Soil Sci. Soc. Am. J.* **60** (1996) 1787–1791.
- [31] CHRISTENSEN, B.T., Physical fractionation of soil and organic matter in primary particle size and density separates, *Adv. Soil Sci.* **20** (1992) 1–90.
- [32] SPYCHER, G., et al., Carbon and nitrogen in the light fraction of a forest soil: vertical distribution and seasonal fluctuations, *Soil Sci.* **135** (1983) 79–87.

## BELOW GROUND NITROGEN IN FABABEAN AND CHICKPEA

D.F. KHAN

Soil and Plant Nutrition Agriculture Research Institute,  
Peshawar, Pakistan

D. CHEN

Institute of Land and Food,  
The University of Melbourne,  
Parkville, Australia

D.F. HERRIDGE, G.D. SCHWENKE

New South Wales Agriculture,  
Tamworth, Australia

M.B. PEOPLES

CSIRO Plant Industry,  
Canberra, Australia

### Abstract

Isotopic and non-isotopic methods were used to quantify below ground nitrogen (BGN) for two winter legumes, fababean (*Vicia faba*) and chickpea (*Cicer arietinum*), under glasshouse and field conditions. In the glasshouse study, estimates of BGN for fababean and chickpea, respectively, were 13 and 10% of total plant N (physical recovery), 11 and 52% (soil  $^{15}\text{N}$  dilution), 30 and 52% (mass N balance), 39 and 53% ( $^{15}\text{N}$ -shoot labelling), 37 and 42% (adjusted  $^{15}\text{N}$  shoot labelling), and 33 and 43 % ( $^{15}\text{N}$  balance). In the field experiment, values were 25 and 77% ( $^{15}\text{N}$ -shoot labelling), 24 and 68% (adjusted  $^{15}\text{N}$  shoot labelling) and 29 and 60% ( $^{15}\text{N}$  balance). When averaged across all estimates (other than physical recovery), BGN of glasshouse-grown plants represented 31% of total plant N for fababean and 48% for chickpea. By comparison, the mean values for BGN as percent of total plant N in the field study using the two methods considered likely to give the most reliable results (adjusted  $^{15}\text{N}$  shoot labelling and  $^{15}\text{N}$  balance) were 27% for fababean and 64% for chickpea.

### 1. INTRODUCTION

The impact of legumes in cereal-based cropping systems can be expressed in a number of ways. One common approach to evaluating legume contributions has been to determine net inputs of fixed  $\text{N}_2$ , calculated by subtracting nitrogen (N) removed in the grain from estimates of the amounts of  $\text{N}_2$  fixed [1, 2]. However, research on legume effects on soil and subsequent cereal crops has revealed an apparent paradox in that measured N benefits from legumes are often greater than might be expected from such N-balance calculations [2]. That the predicted effects of legumes do not coincide with the observed N benefits indicates a deficiency in our understanding of the magnitude of N inputs and subsequent cycling of N in these rotational systems.

Since N-balance determinations have invariably been based on shoot-derived measures of crop N, one area that seems to require further study would be to evaluate the role that nodulated legume roots are playing in the N dynamics of cropping systems [3, 4]. The major inputs of below ground N (BGN) to soil-N pools are likely to come at the end of the growing season as the plants mature and senesce. However, contributions of legume N may also occur throughout the season as roots and nodules die or are sloughed off, and in the form of exudates and secretions (rhizodeposition).

Various techniques have been used to quantify N associated with roots and nodules of legumes. The most simple and common approach has been to physically remove the roots from soil. Given the difficulty and errors associated with such an approach, considerable effort has been directed at development of  $^{15}\text{N}$ -based methodologies. These include growing legumes in  $^{15}\text{N}$ -enriched soil [5] and in situ labelling of shoots with  $^{15}\text{N}$  [3,4]. This paper reports a series of glasshouse and field experiments in which various isotopic and non-isotopic methods were used to quantify BGN of fababean (*Vicia faba*) and chickpea (*Cicer arietinum*).



## 2. MATERIALS AND METHODS

### 2.1. Experimental

#### 2.1.1. Glasshouse studies

The glasshouse experiments were undertaken in a temperature-controlled (27°C day/22°C night), naturally lit glasshouse at CSIRO Plant Industry, Canberra (35°03'S, 147°4'E), ACT, Australia. Eight seeds of each of fababean (cv. Fiord) or chickpea (cv. Moree or Amethyst) were sown into 23-L free-draining pots (28.5 cm diameter × 40 cm deep) containing either 22 kg of a 50:50 mixture of sand and soil, or river sand (in one physical recovery experiment only). The seeds of both species were treated with commercial rhizobial inoculant at sowing. Plants were supplied daily with either N-free nutrient solution or tap water. Ten days after germination, the seedlings were thinned to six per pot. Each treatment consisted of three to five replicated pots. Plants were harvested during late reproductive growth.

#### 2.1.2. Field study

The field experiment was conducted during the 1997 winter at Breeza (31°11'S, 150°25'E) in the northern grain-belt of New South Wales, Australia. The soil was an alkaline (pH 7.4–8.5 in CaCl<sub>2</sub>) Vertosol of heavy clay texture with a total N content (0–10 cm) of 0.175%. The experiment consisted of chickpea (cv. Amethyst) and fababean (cv. Moree) grown in large (30×10 m) plots, replicated four times. At the seedling stage, metal microplot frames, measuring 0.5×0.64 m, were placed in the ground to a depth of about 30 cm in each plot. Each microplot contained either seven (chickpea), or eight (fababean), plants. Both in the glasshouse and field experiments, plants were harvested during late pod-fill prior to the onset of senescence.

### 2.2 Methodologies used to estimate BGN

#### 2.2.1. Physical recovery of roots (glasshouse and field)

Shoots were excised at ground level and either the substrate was removed from pots, or all the soil in replicated 0.32-m<sup>2</sup> microplot areas was dug to a depth of 25 cm. As many fragments of nodulated roots as possible were then removed from the rooting medium. The shoot and recovered roots and nodules were dried at 70°C, weighed and analysed for total N content

#### 2.2.2. Dilution of <sup>15</sup>N-enriched soil (glasshouse)

Shoot residues (15 g) of lupine (*Lupinus albus*) enriched in <sup>15</sup>N (6 atom%) were mixed thoroughly in the soil in each pot. The pots were kept moist and were left in the glasshouse (25°C day/18°C night) for 6 weeks prior to commencement of experimentation, in an attempt to ensure that the <sup>15</sup>N was fully incorporated in the soil organic fraction. Belowground contributions of fixed N were calculated on the basis of the observed 'dilution' of soil <sup>15</sup>N relative to a wheat (*Triticum aestivum*, cv. Janz) or unplanted control (after Ref. [5]). Leachates were collected in drip trays and returned to the pots to minimize losses of <sup>15</sup>N. At plant harvest, shoots were excised, the soil was removed from each pot and roots were recovered. The total soil weight was determined and subsamples collected for later analysis.

#### 2.2.3. N mass balance (glasshouse)

Estimates of legume BGN based on N mass balance were calculated from dry weight determinations and N analyses of collected shoot, root, and soil material from the <sup>15</sup>N-enriched soil experiment described in section 2.2.2.

#### 2.2.4. *In situ* <sup>15</sup>N shoot-labelling (glasshouse and field)

Urea enriched in <sup>15</sup>N (98 atom%) was supplied to the shoot either via a cut petiole (chickpea) or a leaf-flap (fababean) on either three (glasshouse) or five occasions (field) prior to flowering. Each leaf-flap was cut as a narrow “V” underwater with the end of the “V” centred on the mid vein, close to the leaf tip. The leaf-flap or cut petiole was placed in a small tube containing 0.2 mL urea solution and kept in place with a small amount of teristat (blue-tac) putty. This also served to seal the top of the tube to prevent evaporative losses and to attach the tube to a small wooden stake placed next to the plant. Glasshouse plants were fed 8.3 mg <sup>15</sup>N/pot (3×0.2 mL of 0.5% urea solution per plant). In the field experiment, all plants within the microplots were fed 1 mL (5×0.2 mL) of the enriched urea solution (16.1 and 18.4 mg <sup>15</sup>N applied to chickpea and fababean, respectively). The fed petioles and leaves were removed 2 weeks after feeding. Any abscised shoot material was removed within 48 h and retained for analysis. At harvest the shoots were excised, soil was totally removed from pots or field microplots (to 25-cm depth, then cored 25–45 cm), roots were recovered, total soil weight determined, and subsamples collected for analysis. It was assumed that all <sup>15</sup>N excess detected in the soil originated only from <sup>15</sup>N-enriched root material. Estimates of BGN were subsequently calculated from the resulting N and <sup>15</sup>N data using three different approaches.

##### 2.2.4.1. Assuming <sup>15</sup>N enrichments of recovered and unrecovered roots were identical

The first approach assumed that the specific enrichment of recovered root material (i.e. mg <sup>15</sup>N/g root N) was representative of the unrecovered root-derived N still remaining in the soil [3,4].

##### 2.2.4.2. Adjusting for differences in nodulation and <sup>15</sup>N enrichments between recovered and unrecovered roots

Much of the root recovered from soil was derived from the nodulated crown. Since experimentation had determined that nodules were generally depleted in <sup>15</sup>N relative to roots using shoot-labelling methodologies, there was concern that errors would be introduced into the calculations if the unrecovered roots were predominantly unnodulated. Therefore, the <sup>15</sup>N data were adjusted to account for differences in the enrichments of unnodulated root and nodulated root (experimentally determined enrichment ratios were 1:12 for fababean and 1:56 for chickpea) and BGN estimates were recalculated.

##### 2.2.4.3. Above- and belowground distribution of <sup>15</sup>N

The amounts of <sup>15</sup>N label partitioned above and below ground (recovered roots + soil) were calculated, and BGN was estimated on the basis of the assumption of uniform translocation and partitioning of both labelled and unlabelled N to all plant parts.

### 2.3. Analyses and calculations

Plant and soil materials from the glasshouse and field trials were dried, weighed, and roughly ground in a Wiley mill, subsampled, and then finely ground with a ring grinder. The total N and <sup>15</sup>N contents of the dried, ground samples were determined by combustion using an automatic N and C analyser interfaced with a 20-20 stable isotope mass spectrometer (Europa Scientific). The <sup>15</sup>N data were expressed as δ<sup>15</sup>N or parts per thousand (‰) relative to <sup>15</sup>N composition of atmospheric N<sub>2</sub> (i.e. 0.3663 atom% <sup>15</sup>N) using the following equation:

$$\delta^{15}\text{N} = 1000 \times \frac{(\text{atom}\% \text{ } ^{15}\text{N}_{\text{sample}} - 0.3663)}{0.3663}$$

The content of excess <sup>15</sup>N (enrichment above natural abundance) was calculated by comparing the <sup>15</sup>N composition of enriched plant and soil samples with matching natural abundance material (unenriched controls).

### 3. RESULTS

#### 3.1. Glasshouse studies

##### 3.1.1. Physical recovery of roots

Estimates of BGN based on the physical recovery of roots from the potting mix were made on three occasions. The amount of N recovered in roots and nodules represented between 9 and 12% of total plant N (mean 10%) for chickpea and between 10 and 19% (mean 13%) for fababean (Table I). The relative importance of nodule N appeared to differ between the two species, with nodules contributing a much higher proportion of BGN of chickpea (65%) compared to fababean (26%).

##### 3.1.2. Dilution of $^{15}\text{N}$ -enriched soil

Comparison of the  $^{15}\text{N}$  enrichments of the legume shoots (chickpea 545‰, fababean 210‰), with the wheat control (3,976‰) indicated significant contributions of fixed N for growth (the proportion of legume N derived from  $\text{N}_2$  fixation, %Ndfa [3], was calculated to be 86 and 95% for chickpea and fababean, respectively). It was, therefore, surprising to find that the enrichment of the fababean soil (452‰) was similar to that of wheat soil, the non- $\text{N}_2$ -fixing control (454‰). It was, however, lower than that measured in soil in the unplanted pot (513‰). In contrast to this observation, the low level of enrichment detected in the chickpea soil (401‰) implied dilution of  $^{15}\text{N}$  by root-derived fixed N.

Belowground N of the two legumes was estimated by the  $^{15}\text{N}$  soil-dilution method proposed by Poth et al. [5]. The  $^{15}\text{N}$  enrichments of soil in the legume pots were related to the enrichment of the wheat soil to determine % soil N derived from  $\text{N}_2$  fixation. The Pfix values determined for the legumes were then used to adjust the values of soil N derived from  $\text{N}_2$  fixation to root-derived N in soil. Estimates of BGN calculated in this way (Table I) ranged from 11% (fababean) to 52% (chickpea).

##### 3.1.3. N mass balance

Data from the enriched-soil study were used to construct N budgets for both legume species. Soil N present in each pot at the beginning of the experiment was subtracted from soil N measured at final harvest to determine any net change during the course of plant growth. This was added to N measured in the recovered roots to determine BGN. Values were calculated to be 30 and 52% for fababean and chickpea, respectively (Table I).

##### 3.1.4. In situ $^{15}\text{N}$ shoot-labelling

Recovery of fed  $^{15}\text{N}$  in the shoot, roots, and soil ranged from 76% (chickpea) to 90% (fababean). The  $^{15}\text{N}$  abundance of the potting mix was significantly enriched following shoot-labelling of fababean (42‰) and chickpea (53‰) compared to soil in the untreated controls (3‰).

TABLE I. ESTIMATES OF BELOWGROUND N AS A PERCENTAGE OF TOTAL PLANT N FOR GLASSHOUSE- AND FIELD-GROWN FABABEAN AND CHICKPEA

Species	Study	Method					
		Physical recovery	Soil $^{15}\text{N}$ dilution	Mass N balance	$^{15}\text{N}$ shoot labelling	Adjstd $^{15}\text{N}$ shoot lablg	$^{15}\text{N}$ balance
Fababean	Glasshouse	13	11	30	39	37	33
	Field	2	—	—	25	24	29
Chickpea	Glasshouse	10	52	52	53	42	43
	Field	7	—	—	77	68	60

The  $^{15}\text{N}$  enrichments of the crown roots (including crown root, tap root and major laterals), and distal roots (minor laterals and fine roots) were identical (355‰) for fababean, but differed in the case of chickpea (crown roots, 317‰; distal roots, 241‰).

Assuming that the  $^{15}\text{N}$  and N characteristics of the recovered root material was representative of the unrecovered root-derived N remaining in the soil [3,4], BGN was calculated to represent 39% and 53% of total plant N for fababean and chickpea, respectively (Table I). Estimates of BGN were similar for fababean (37%), and slightly lower for chickpea (42%) if it was assumed that the unrecovered roots were unnodulated and the data were adjusted to account for the likely difference in enrichment between nodulated and unnodulated roots (Table I). Values were again similar (33% for fababean and 43% for chickpea, Table I) if it was assumed that the percentage distribution of  $^{15}\text{N}$  label measured in the shoots, recovered roots, and soil reflected the above- and belowground partitioning of plant N.

### 3.2. Field study

#### 3.2.1. Physical recovery of roots

It was exceedingly difficult to recover intact root fragments from the heavy textured soil (58% clay, 22% silt, 20% sand) at the field site. The amounts of N present in the roots removed from the soil (0.13 and 0.16 g N per microplot for fababean and chickpea, respectively) were dwarfed by the amounts of N measured in the shoots (5.52 and 2.18 g N for fababean and chickpea, respectively). Subsequent estimates of BGN represented only 2% (fababean) to 7% (chickpea) of the total plant N recovered (Table I).

#### 3.2.2. *In situ* $^{15}\text{N}$ shoot-labelling

Recovery of  $^{15}\text{N}$  in harvested plant parts and soil accounted for 91 to 92% of the  $^{15}\text{N}$  enriched urea-N applied. Enrichments in the 0- to 25- and 25- to 45-cm layers of soil removed from the microplots were 18‰ and 8.7‰ under fababean, and 30‰ and 8.8‰ under chickpea, respectively, compared with 6.1 to 6.3‰ in soil collected from outside the microplots. The enrichments of fababean material sampled from the microplots were 568‰ (shoot) and 674‰ (root), and the values for chickpea were 705‰ (shoot) and 331‰ (root) compared to 0 to 3.5‰ in plants sampled from the surrounding unenriched crop. Calculations using the relative  $^{15}\text{N}$  excess of the recovered roots and the  $^{15}\text{N}$  enrichment of soil indicated that BGN represented 25% of total crop N for fababean and 77% for chickpea (Table I). The high BGN value for chickpea was caused by a combination of a low  $^{15}\text{N}$  enrichment of recovered roots and a relatively high enrichment of the 0 to 25 cm soil N. Neither the 'adjusted' nor ' $^{15}\text{N}$  balance' approaches had a large effect on estimates of BGN for fababean (24 and 29%, Table I), but both modifications of the shoot-labelling technique reduced the values calculated for chickpea (68 and 60%, Table I).

## 4. DISCUSSION

It appears from Table I that the most error-prone and inaccurate method for estimating BGN is the physical recovery of roots. The values obtained with physical recovery (10–13% of whole plant N in the glasshouse, and 2–7% in the field) were only a fraction of those obtained using the other methodologies (Table I). This should be expected since even if it were possible to completely recover intact root systems, such measures would not include N derived from the turnover of nodules and roots or root exudations that occur during growth.

With the exception of the soil  $^{15}\text{N}$ -dilution method for fababean, most techniques used in the glasshouse studies gave reasonably similar determinations of BGN (Table I). Averaged across all estimates (other than physical recovery), BGN of glasshouse-grown plants represented 30% of total plant N for fababean and 48% for chickpea.

Although the  $^{15}\text{N}$ -based methods used in the field study have questionable assumptions with built-in errors, it was reassuring that all three calculations provided estimates that were similar to each other

(24–29% and 26% mean for fababean, 60–77% and 68% mean for chickpea), and were comparable to those obtained under very different conditions in the glasshouse (Table I). However, it is unlikely that there is a single value for BGN for a species, and it is reasonable to assume that the root:shoot ratio is influenced by growth conditions or stress and for species to respond in differing ways. This presumably explains why estimates of BGN for chickpea in the field were slightly higher than detected in the glasshouse (Table I).

## 5. CONCLUSION

Field and glasshouse studies of fababean and chickpea indicated that much higher proportions of total legume N are associated with, or derived from, roots than previously believed. It is clear that BGN represents an important pool of residual N that has been grossly underestimated or ignored in past calculations of rotational N budgets.

## ACKNOWLEDGEMENTS

This research would not have been possible without the resources provided by various agencies (NSW Agriculture, CSIRO, University of Melbourne) and external funding via the John Allwright Fellowship and Australian Centre for International Agricultural Research (ACIAR).

## REFERENCES

- [1] CHALK, P.M., Dynamics of biologically fixed N in legume-cereal rotations, *Aust. J. Agric. Res.* **49** (1998) 303–316.
- [2] PEOPLES, M.B., et al., Biological nitrogen fixation: an efficient source of nitrogen for sustainable agricultural production? *Plant Soil* **174** (1995) 3–28.
- [3] ROCHESTER, I.J., et al., Faba beans and other legumes add nitrogen to irrigated cotton cropping systems, *Aust. J. Expl. Agric.* **38** (1998) 253–260.
- [4] RUSSELL, C.A., FILLERY, I.R.P., Estimates of lupin belowground biomass nitrogen, dry matter and nitrogen turnover to wheat, *Aust. J. Agric. Res.* **47** (1996) 1047–1059.
- [5] POTH, M., et al., Quantification by direct <sup>15</sup>N dilution of fixed N<sub>2</sub> incorporation into soil by *Cajanus cajan* (pigeon pea), *Soil Biol. Biochem.* **18** (1986) 125–127.

## SEASONAL VARIABILITY OF SOIL CO<sub>2</sub> FLUX AND ITS ISOTOPIC COMPOSITION ( $\delta^{13}\text{C}$ , $\delta^{14}\text{C}$ , $\delta^{18}\text{O}$ )

Z. GORCZYCA, T. KUC, K. ROZANSKI  
Faculty of Physics and Nuclear Techniques,  
University of Mining and Metallurgy,  
Krakow, Poland

### Abstract

The flux and isotopic composition of soil CO<sub>2</sub> were monitored at three sites in southern Poland, from January 1998 to January 2000. The sites represent typical ecosystems of central Europe: (i) mixed forest; (ii) a cultivated agricultural field, and (iii) grassland. Methods based on the inverted-cup principle were used. The flux of soil CO<sub>2</sub> revealed distinct seasonal fluctuations, with maximum values up to approximately 30 mmol/m<sup>2</sup>/h during the summer months and around 90% lower during winter. Also, significant differences among the monitored sites were detected; the flux density was highest for the mixed forest site and about 50% lower for the cultivated grassland. Carbon-13 content of the soil CO<sub>2</sub> revealed little seasonal variability, with  $\delta^{13}\text{C}$  values essentially reflecting the isotopic composition of the soil organic matter and the vegetation type. The  $\delta^{14}\text{C}$  content of soil CO<sub>2</sub> flux revealed slight seasonality, with lower  $\delta^{14}\text{C}$  values during winter. Significantly lower  $\delta^{14}\text{C}$  values were recorded at depth.

### 1. INTRODUCTION

Isotopes of carbon ( $^{13}\text{C}$ ,  $^{14}\text{C}$ ) are useful tools for studying the global C cycle. They provide additional information for currently used models of the C cycle and help to characterize sources of, and sinks for, C, both on regionally and globally [1,2]. The amount of  $^{18}\text{O}$  in atmospheric CO<sub>2</sub> delivers additional information about fluxes between the continental biosphere (soils and plant cover) and the atmosphere [3,4,5]. Whereas the monitoring networks for studying isotopic variability of atmospheric CO<sub>2</sub> are relatively well developed, the relevant data for soil CO<sub>2</sub> fluxes, which constitute an important component of the C cycle on continents, are still fragmentary. Studies on  $^{13}\text{C}$  and  $^{14}\text{C}$  composition of soil CO<sub>2</sub> were carried out in the 1980s in Germany [6,7]. Stable-isotope composition of soil CO<sub>2</sub> ( $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$ ) was monitored for a period of 1 year in Switzerland by Hersterberger and Siegenthaler [8]. A comprehensive characterization of the seasonal variability of both soil CO<sub>2</sub> flux entering the atmosphere and its isotopic composition ( $\delta^{13}\text{C}$ ,  $\delta^{14}\text{C}$ ,  $\delta^{18}\text{O}$ ) has not been attempted so far. This paper presents preliminary results obtained from a 2-year study (January 1998–January 2000) in southern Poland.

### 2. METHODS

The flux and isotopic composition of soil CO<sub>2</sub> were monitored at sites that represent common ecosystems in central Europe: mixed forest, an arable field, and cultivated grassland.

The mixed forest contains oak, hornbeam, and pine, and the sampling site was approximately 40 km east of Krakow (20°23'E, 50°04'N) on a sandy soil, low in organic matter. The agricultural site is located in a village approximately 150 km southeast of Krakow (19°56'E, 50°03'N), in the foothills of the Carpathians, in a typical field where potatoes were grown during the reported time period. The cultivated grassland site, located in the same village, belongs to a lower class according to the common agricultural classification. The meadow where the sampling unit was placed has been in existence for at least 30 years.

To monitor the flux of soil CO<sub>2</sub> and its isotopic composition, two versions of the inverted-cup principle were used: (i) the closed-system version, allowing collection of monthly-averaged samples of soil CO<sub>2</sub> for  $^{14}\text{C}$  and  $^{13}\text{C}$  determinations, and (ii) the in-growth version allowing the apparent values of the CO<sub>2</sub> flux and its isotopic composition ( $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$ ) to be determined. In addition, depth

profiles of the soil air were regularly collected to determine CO<sub>2</sub> concentration and its isotopic composition ( $\delta^{13}\text{C}$ ,  $\delta^{14}\text{C}$ ).

## 2.1. The inverted-cup method

The sampling system consisted of a large metal container (volume ca. 40 L), equipped with appropriate connections. It was placed on the soil surface (Fig. 1), thus allowing accumulation of emitted gases. To limit direct contact with the outside atmosphere, the container was hammered about 10 cm into the soil. This system can be used in two different sampling modes, depending on the type of sample being collected.

For determination of the soil CO<sub>2</sub> flux and its isotopic composition ( $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$ ), air from the container was drawn using a diaphragm pump through a trap filled with a drying agent (cf. Fig. 1a) at specified time intervals, and several subsequent samples of air were collected in glass flasks (ca. 0.5 L). The CO<sub>2</sub> concentrations in the samples were determined in the laboratory using gas chromatography.

To collect samples of soil CO<sub>2</sub> for radiocarbon analysis, air from the container was circulated through a trap filled with a molecular sieve to which CO<sub>2</sub> was adsorbed (Fig. 1b). A dedicated electronic control system switched the diaphragm pump on and off at programmed time intervals, covering uniformly the entire sampling period (1 month). Periods of pumping were relatively short, thus allowing near-equilibrium conditions under the container. The system was operated by a car battery, and was essentially maintenance-free, except for changing the trap containing the molecular sieve. The collected CO<sub>2</sub> was extracted from the molecular sieve in the laboratory and its <sup>14</sup>C activity was measured using benzene synthesis and liquid scintillation spectrometry. Carbon-13 content of the extracted CO<sub>2</sub> was analysed with a mass spectrometer.

## 2.2. Depth profiles

Information about the flux and isotopic composition of the soil CO<sub>2</sub> was obtained also through sampling the soil air at various depths of the soil column. The experimental set-up was as shown in Fig. 1c. In order to obtain profiles of CO<sub>2</sub> concentration, the soil air from various depths was drawn into glass flasks (ca. 0.5 L) using a diaphragm pump. After measuring CO<sub>2</sub> concentration by gas chromatography, CO<sub>2</sub> was cryogenically extracted from the remaining samples in a special vacuum line [9] and <sup>13</sup>C and <sup>18</sup>O contents were measured.

For <sup>14</sup>C analysis of resident soil CO<sub>2</sub>, approximately 15 L of air were drawn from the given depth and pumped into special sampling bags made of polyvinyl fluoride. In the laboratory, CO<sub>2</sub> was extracted cryogenically in a vacuum line and its concentration was calculated by the volumetric method (the ratio of the CO<sub>2</sub> volume to the volume of air pumped out of the bag). The <sup>14</sup>C activity of the purified gas was then measured in miniature gas proportional counters [10].

## 2.3. Soil CO<sub>2</sub> flux

The flux of soil CO<sub>2</sub> into the atmosphere was determined with the aid of the in-growth method. The CO<sub>2</sub> concentration was measured inside a newly established inverted cup as a function of time. The flux density of the soil CO<sub>2</sub> into the atmosphere was then calculated from the well known formula:

$$j_x = \frac{V \cdot \left. \frac{\partial C}{\partial t} \right|_{t=0}}{S} \quad (1)$$

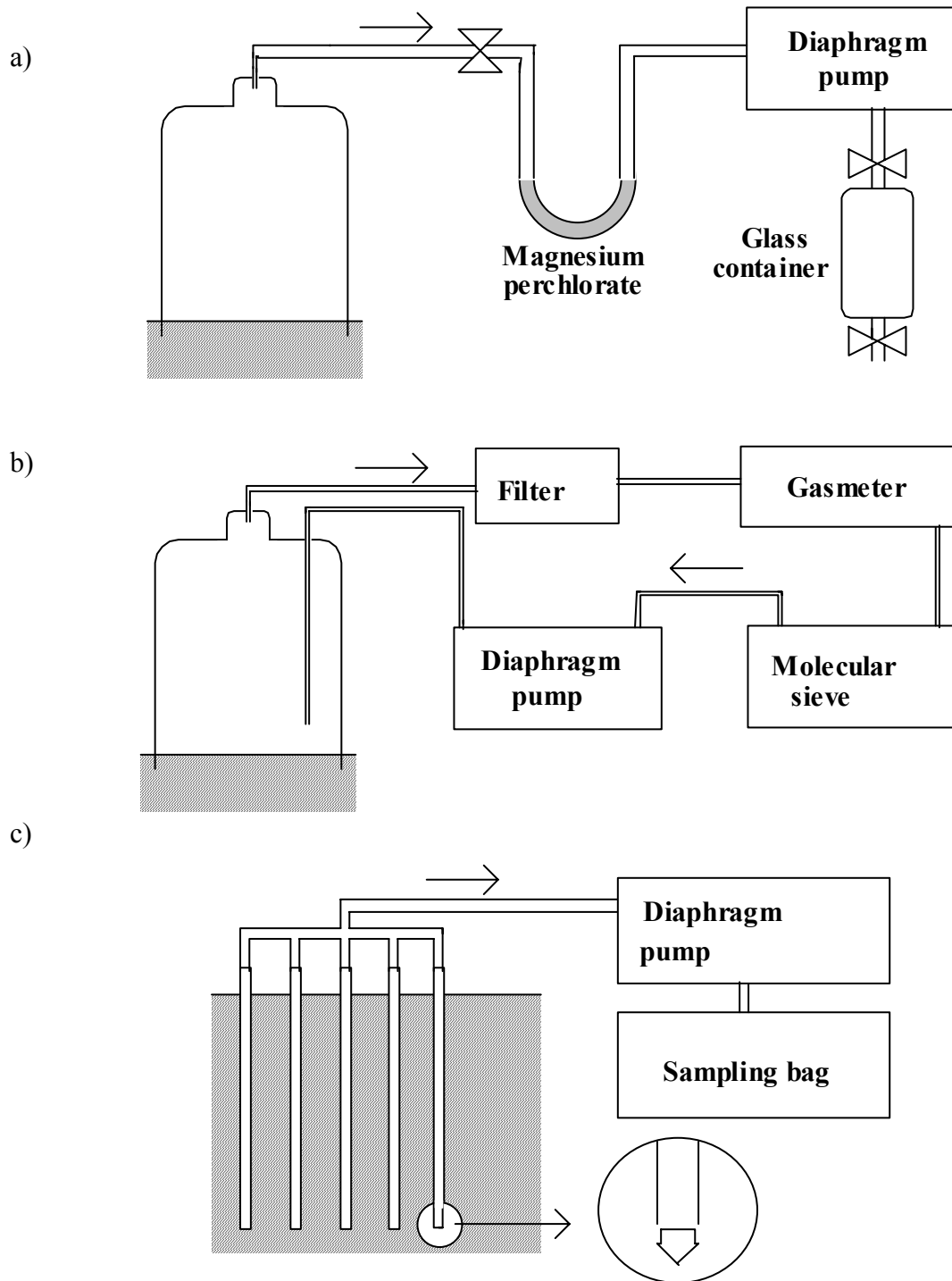


FIG. 1. Schematic representation of three modes of sampling to assess flux and isotopic composition of CO<sub>2</sub> released from soil: (a) the in-growth method, (b) for radiocarbon analysis, (c) for depth profiles of CO<sub>2</sub> concentration and its isotopic composition.

where

$V$  is the volume under the inverted cup (m<sup>3</sup>),

$S$  surface of soil covered by the inverted cup (m<sup>2</sup>)

and  $\partial C/\partial t$  is the change of CO<sub>2</sub> concentration (ppmv/s) under the inverted cup at  $t=0$ , derived from the experimental data (CO<sub>2</sub> concentration of air samples collected from under the inverted cup at regular time intervals). The temporal change of CO<sub>2</sub> concentration inside the inverted cup (cf. Fig. 2a) can be described by the following equation:



$$C(t) = \frac{A}{B} \cdot (1 - e^{-B \cdot t}) + C_0 \quad (2)$$

where

A and B are fitting parameters (1/s)

and  $C_0$  is the atmospheric  $\text{CO}_2$  concentration (ppmv).

It should be noted that the in-growth method can be used also for other trace gases released from the soil (e.g.  $\text{N}_2\text{O}$ ).

## 2.4. Isotopic composition of the soil $\text{CO}_2$ flux ( $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ )

The in-growth method described above was also used to obtain the isotopic composition of the soil  $\text{CO}_2$  flux ( $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$ ). Once the inverted cup is installed,  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  of  $\text{CO}_2$  under it undergo gradual changes due to mixing of atmospheric  $\text{CO}_2$  initially present inside the cup, as the  $\text{CO}_2$  diffuses from the soil (cf. Fig. 2b, c). To the first approximation, this process can be considered as a two-component mixing. Consequently, it can be shown that when  $\delta^{13}\text{C}$  ( $\delta^{18}\text{O}$ ) values are plotted as a function of the reciprocal of  $\text{CO}_2$  concentration inside the cap, they should form a straight line (Fig. 3a, b). The extrapolation of the fitted line to  $1/C$  equal zero yields the  $\delta^{13}\text{C}$  ( $\delta^{18}\text{O}$ ) value of the soil  $\text{CO}_2$ .

## 3. RESULTS AND DISCUSSION

The following analyses were performed during the period January 1998 to January 2000 at the selected sites: (i) monthly mean  $\text{CO}_2$  equilibrium concentration in the soil, supplemented by spot determinations of the soil  $\text{CO}_2$  flux using the in-growth method, (ii)  $^{13}\text{C}$  and  $^{18}\text{O}$  content in the soil  $\text{CO}_2$  flux at three selected sites, (iii)  $^{14}\text{C}$  and  $^{13}\text{C}$  content in monthly averaged samples of soil  $\text{CO}_2$  collected in molecular sieve, and (iv)  $^{14}\text{C}$  and  $^{13}\text{C}$  content in  $\text{CO}_2$  collected from different depths at two selected sites, representing summer and winter conditions.

Figure 4 shows the monthly averaged  $\text{CO}_2$  concentration inside the inverted cup as a function of time at the three sites. The average  $\text{CO}_2$  concentration was derived from the volume of  $\text{CO}_2$  collected in the molecular sieve trap and the volume of air pumped through the trap in the given month. This measured  $\text{CO}_2$  concentration should closely represent the equilibrium concentration in the upper soil zone. It is apparent from the figure that the equilibrium concentration of  $\text{CO}_2$  in the soil depends on the type of ecosystem studied, and season of the year. The highest values were observed for the agricultural field where potatoes were grown (up to 1.4% vol.), compared with ca. 0.8% vol. observed at the same time for the cultivated grassland.

The flux of soil  $\text{CO}_2$ , linked to the equilibrium soil  $\text{CO}_2$  concentration, was derived from spot measurements at the selected sites using the in-growth method. It also revealed distinct seasonal fluctuations, with maximum values up to ca. 30 mmol/m<sup>2</sup>/h during the summer months and around a tenth of these values during winter (Table I). Also, significant differences among the monitored sites were noticed. The  $\text{CO}_2$  flux density was highest for the mixed forest site and approximately 50% less for the cultivated grassland. These values for  $\text{CO}_2$  flux densities at the soil-atmosphere interface are consistent with measurements obtained in Germany [7] and in Switzerland [8].

Seasonal differences in the isotope characteristics of soil  $\text{CO}_2$  flux at the three sites are summarized in Table I. Carbon-13 content of the soil  $\text{CO}_2$  revealed little seasonal variability, with  $\delta^{13}\text{C}$  values essentially reflecting the isotopic composition of the soil organic matter and the vegetation type. It is noteworthy that  $\delta^{13}\text{C}$  values obtained for the  $\text{CO}_2$  flux from the soil at a given site differed from those for the resident  $\text{CO}_2$  concentrations in the soil. This difference, reaching several per mil (resident  $\text{CO}_2$  being enriched in  $^{13}\text{C}$ ), is understood in the light of kinetic fractionation associated with diffusion of  $\text{CO}_2$  from the soil [e.g. 6].

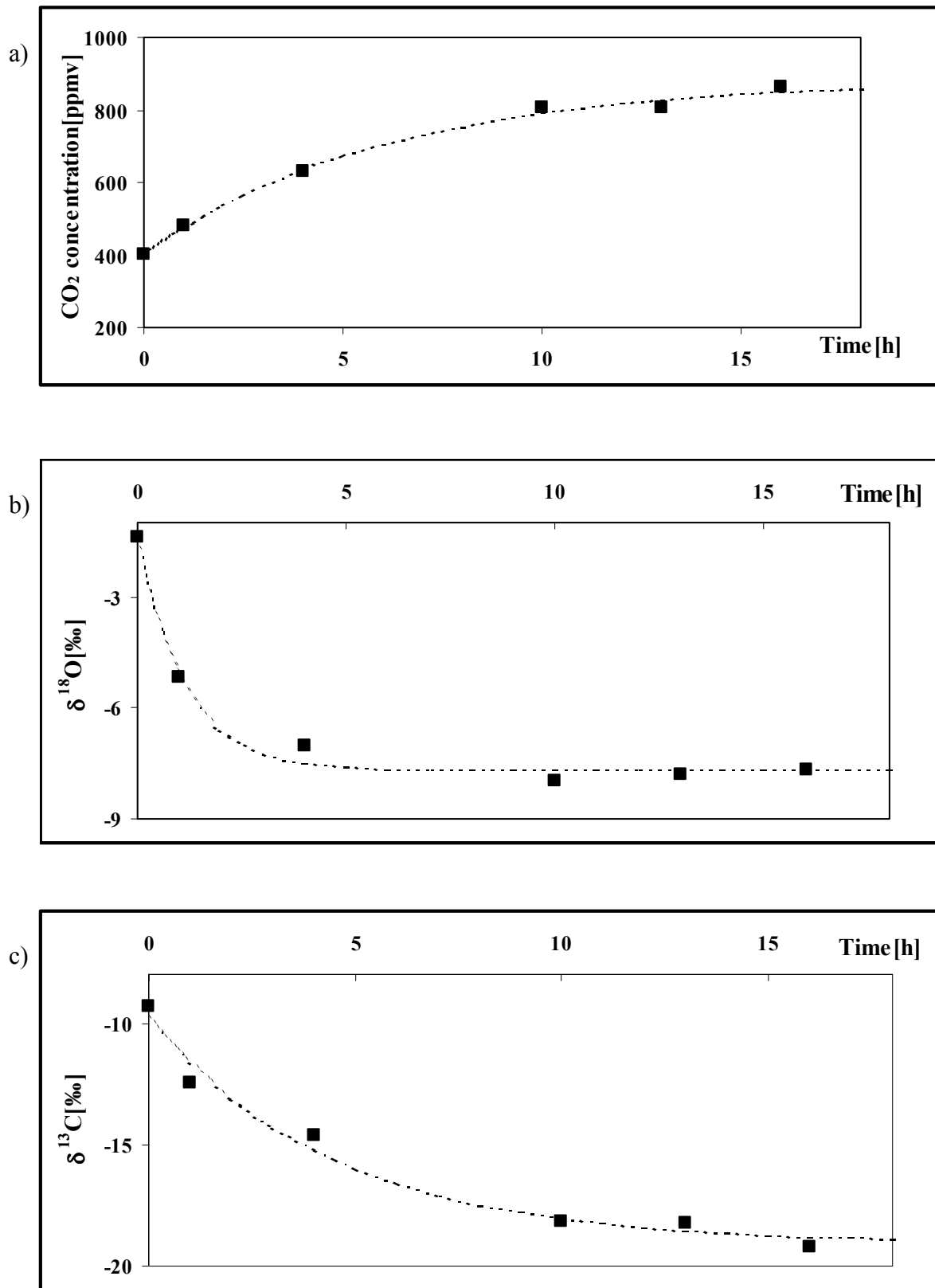


FIG.2. Typical evolution of CO<sub>2</sub> concentration and its isotopic composition ( $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$ ) inside the inverted cup, as a function of time elapsed since setting up the sampling container on the soil surface. Data represent the agricultural field site sampled on February 12, 1999.

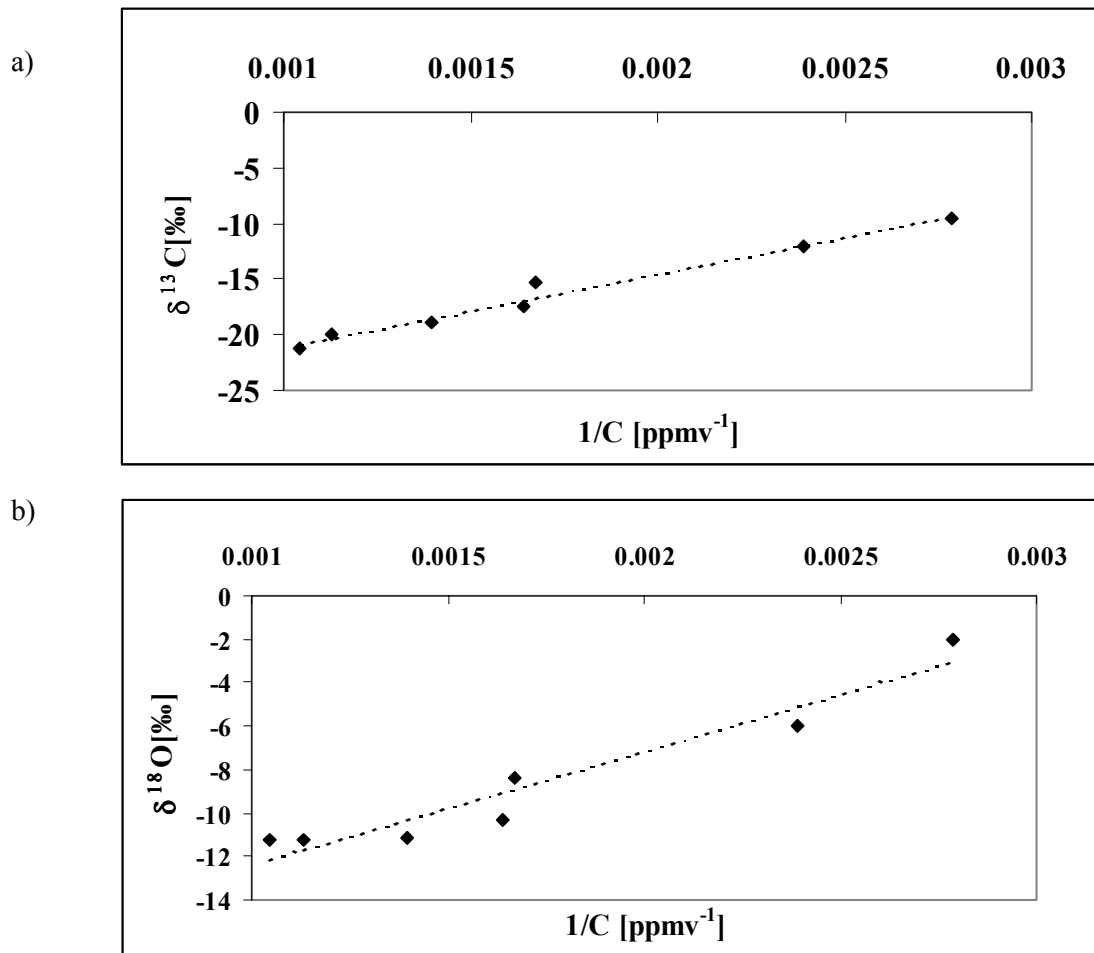


FIG. 3.  $\delta^{13}\text{C}$  and  $\delta^{18}\text{O}$  values of  $\text{CO}_2$  inside the inverted cup, plotted versus reciprocal of the  $\text{CO}_2$  concentration (forest site, Jan. 28, '99). The linear approximation (least square fit) of the data points yields the isotopic composition of the soil  $\text{CO}_2$  flux:  $\delta^{13}\text{C} = -27.6 \pm 0.87\text{‰}$ ,  $\delta^{18}\text{O} = -17.6 \pm 1.21\text{‰}$ .

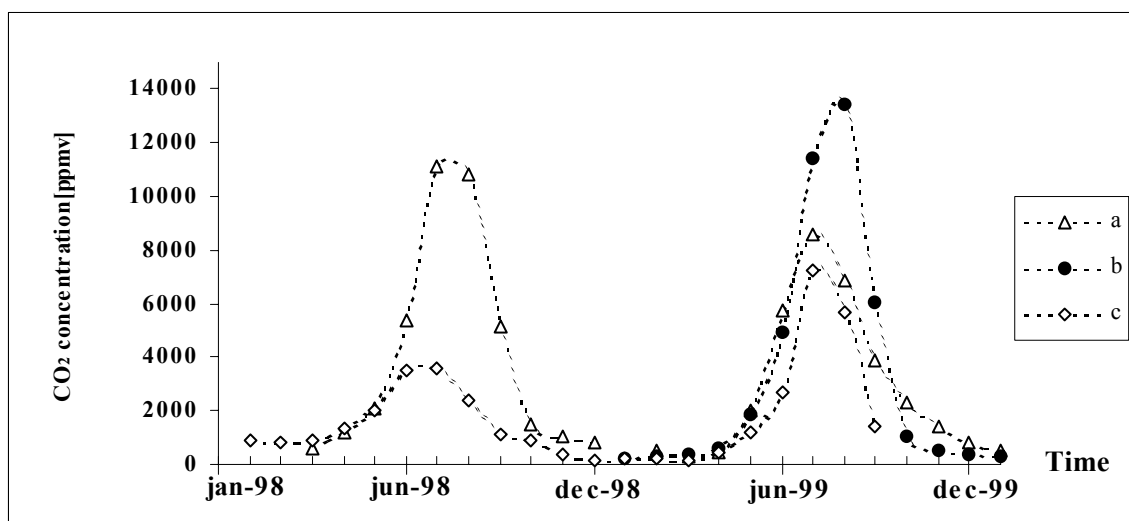


FIG.4. Seasonal changes of soil  $\text{CO}_2$  equilibrium concentration measured at three sites in southern Poland: (a) mixed forest, (b) agricultural field, and (c) grassland.

TABLE I. AVERAGE CHARACTERISTICS OF THE SOIL CO<sub>2</sub> FLUX ENTERING THE ATMOSPHERE IN SUMMER (S) AND WINTER (W), AS MEASURED AT THREE REPRESENTATIVE SITES IN SOUTHERN POLAND, JANUARY 1998 TO JANUARY 2000

Characteristic		Mixed forest	Agricultural field	Cultivated grassland
Soil CO <sub>2</sub> flux into the atmosphere				
Flux density at the surface (mmol/m <sup>2</sup> /h) <sup>a</sup>	S	22.0	15.2	11.8
	W	2.8	1.4	1.5
Carbon-13 content (δ <sup>13</sup> C <sub>V-PDB</sub> ) (‰) <sup>a</sup>	S	28.6	-28.6	-28.8
	W	-28.0	-27.0	—
Carbon-14 content (δ <sup>14</sup> C) (‰) <sup>b</sup>	S	163	148	158
	W	144	97	144
Soil CO <sub>2</sub> at depth (40 cm)				
CO <sub>2</sub> concentration (ppmv)	S	5,610	37,420	17,055
	W	1,430	8,860	5,110
Carbon-13 content (δ <sup>13</sup> C <sub>V-PDB</sub> ) (‰)	S	-21.7	-26.1	-25.3
	W	-19.5	-25.6	—
Oxygen-18 content (δ <sup>18</sup> O <sub>V-PDB</sub> ) (‰)	S	-6.1	-10.3	-8.0
	W	-7.5	-5.2	-8.1
Carbon-14 content (δ <sup>14</sup> C) (‰) <sup>b</sup>	S	150	-2.0	71
	W	105	-31	-60

<sup>a</sup>Derived using the in-growth method.

<sup>b</sup>Radiocarbon content is reported as δ<sup>14</sup>C (per mil deviation from the <sup>14</sup>C standard representing 100% of modern C, without δ<sup>13</sup>C correction).

The <sup>18</sup>O isotope composition of soil CO<sub>2</sub> flux leaving the surface showed remarkable seasonality, with less-negative δ<sup>18</sup>O values recorded during the summer months. This is most likely due to seasonally varying temperature in the uppermost layers of the soil, influencing the equilibrium fractionation factor between soil water and CO<sub>2</sub>. Also, seasonal fluctuations of δ<sup>18</sup>O of the soil moisture in this region, caused both by the seasonality of <sup>18</sup>O in rainfall and by enhanced evaporation of soil water during summer, may contribute to the observed effect.

The <sup>14</sup>C content of soil CO<sub>2</sub> flux also exhibited slight seasonality, with generally lower δ<sup>14</sup>C values recorded during winter. Also, significantly lower δ<sup>14</sup>C values were recorded at depth (40 cm), reaching -60 per mil for the cultivated grassland.

#### 4. CONCLUSIONS

The flux of soil CO<sub>2</sub> revealed distinct seasonal fluctuations, with maximum values of approximately 30 mmol/m<sup>2</sup>/h during the summer months and 90% lower values recorded during winter. Another important factor is the type of land cover. Differences of up to a factor of two were recorded between the investigated sites, which represented common ecosystems in central Europe: mixed forest; an agricultural field and cultivated grassland.

Carbon-13 content of the soil CO<sub>2</sub> revealed little seasonal variability, with δ<sup>13</sup>C values essentially reflecting the isotopic composition of the soil organic matter and the vegetation type. The other

isotope characteristics of the soil CO<sub>2</sub> flux ( $\delta^{14}\text{C}$ ,  $\delta^{18}\text{O}$ ) varied with season, with lower  $\delta^{14}\text{C}$  values and more negative  $\delta^{18}\text{O}$  values recorded during winter.

## REFERENCES

- [1] BATTLE, M., et al., Global carbon sinks and their variability inferred from atmospheric O<sub>2</sub> and  $\delta^{13}\text{C}$ , *Science* **287** (2000) 2467–2470.
- [2] KUC, T., ZIMNOCH, M., Changes of the CO<sub>2</sub> sources and sinks in a polluted urban area (southern Poland) over the last decade, derived from the carbon isotope composition, *Radiocarbon* **40** (1998) 417–423.
- [3] FARQUHAR, G.D., et al., Vegetation effects on the isotope composition of oxygen in atmospheric CO<sub>2</sub>, *Nature* **363** (1993) 439–443.
- [4] CIAIS, P., et al., A three-dimensional synthesis study of  $\delta^{18}\text{O}$  in atmospheric CO<sub>2</sub>. 1. Surface fluxes, *J. Geophys. Res.* **102D** (1997) 5857–5872.
- [5] CIAIS, P., et al., A three-dimensional synthesis study of  $\delta^{18}\text{O}$  in atmospheric CO<sub>2</sub>. 2. Simulations with the TM2 transport model, *J. Geophys. Res.*, **102D** (1997) 5873–5883.
- [6] DÖRR, H., MÜNNICH, K.O., Carbon-14 and carbon-13 in soil CO<sub>2</sub>, *Radiocarbon* **22** (1980) 909–918.
- [7] DÖRR, H., MÜNNICH, K.O., Annual variation in soil respiration in selected areas of the temperate zone, *Tellus* **39B** (1987) 114–121.
- [8] HERSTERBERGER, R., SIEGENTHALER, U., Production and stable isotopic composition of CO<sub>2</sub> in a soil near Bern, Switzerland, *Tellus* **43B** (1991) 197–205.
- [9] MIROSLAW, J., et al., Isotopic composition of CO<sub>2</sub> in the heavily polluted urban atmosphere and in the mountain area (Southern Poland),” *Isotope Techniques in the Study of Past and Current Environmental Changes in the Hydrosphere and the Atmosphere*, IAEA, Vienna (1997) 37–48.
- [10] GORCZYCA, Z., et al., Gas counting system for <sup>14</sup>C dating of small samples in the Kraków laboratory, *Radiocarbon* **40** (1998) 129–135.

# **CAN THE $^{13}\text{C}$ NATURAL ABUNDANCE TECHNIQUE BE APPLIED TO QUANTIFY THE SOIL ORGANIC MATTER IN CROP ROTATIONS WITH MIXED $\text{C}_3$ AND $\text{C}_4$ PLANTS?**

S.D. WANNIARACHCHI\*, R.P. VORONEY  
Department of Land Resource Science,  
University of Guelph, Guelph, Ontario, Canada

## **Abstract**

In this study, the  $^{13}\text{C}$  natural abundance technique was used to quantify the sources of soil C in a mixed  $\text{C}_3$  and  $\text{C}_4$  system containing corn ( $\text{C}_4$ ) and forages (alfalfa and brome grass,  $\text{C}_3$ ). Corn-derived C in soil was estimated using a mixing model as previously. The quantity of forage-derived C in soil was estimated using two independent methods for comparison, with assumptions that there is no differential decomposition and stabilization of soil C under monoculture compared to rotations. The subtraction method yielded higher values for forage-derived C than the mixing-model method, which is based on isotopic signatures. The observed differences in forage-derived C estimates may be largely due to spatial variability of soil C at the study site. Further research with proper field layout and crop selection is required to select and refine the best approach. This study shows the potential for using the  $\delta^{13}\text{C}$  technique in mixed  $\text{C}_3$ - $\text{C}_4$  systems to quantify contributing C inputs to soil.

## **1. INTRODUCTION**

The natural  $^{13}\text{C}$  abundance ( $\delta^{13}\text{C}$ ) of soil can be used as an in-situ labelling technique for soil organic matter (SOM) studies under field conditions [1]. The  $\delta^{13}\text{C}$  technique has been applied mainly to determine whole-SOM dynamics after a shift in vegetation from  $\text{C}_3$  to  $\text{C}_4$ , or vice versa. However, due to increasing concerns over soil quality and sustainable management,  $\text{C}_4$  crops are commonly grown in rotation with  $\text{C}_3$  crops. Huggins et al. [2] estimated corn-derived C in SOM of corn-soybean rotations; however, no attempt was made to estimate the soybean-derived C ( $\text{C}_3$ ). Rao et al. [3] and Cadisch and Giller [4] estimated legume contributions to SOM under mixed tropical pastures ( $\text{C}_4$ ) and legume ( $\text{C}_3$ ) systems using  $\delta^{13}\text{C}$  techniques. In the latter two studies, contrasting approaches were used, and more research is required to evaluate and select  $\delta^{13}\text{C}$  techniques appropriate to study SOM dynamics under mixed  $\text{C}_3$  and  $\text{C}_4$  systems. Therefore, our objective was to evaluate the adaptability of  $\delta^{13}\text{C}$  techniques to estimate forage-derived C in the soil of a corn-forage rotation.

## **2. MATERIALS AND METHODS**

### **2.1. Site description, soil sampling, and preparation**

Soil samples were taken from a field experiment located at the Elora Research Station of the University of Guelph, Elora (43°52'N, 80°21'W), Ontario. A corn-forage rotation experiment had been in progress since 1980, three treatments of which were selected for the present study on SOM: continuous corn (grain corn for 16 years), a corn-brome grass rotation (9 years of brome grass and 6 years of corn), and a corn-alfalfa rotation (9 years of alfalfa and 6 years of corn). All selected treatments were under conventional tillage, except for the duration of forage cropping.

The site was sampled in the summer of 1995, to 50 cm using a core sampler. Soil cores were cut in the field into segments, 0 to 5, 5 to 10, 10 to 15, 15 to 20, 20 to 25, 25 to 30, 30 to 40, and 40 to 50 cm, which were pooled to make composite samples per depth zone per replicate plot. The experiment had a randomized complete block design with four replicates. Soil from a nearby native forest site was sampled to use as a background for  $\delta^{13}\text{C}$  calculations.

---

\*Present address: Faculty of Agriculture, University of Ruhuna, Kamburupitiya 81100, Sri Lanka.

All soil samples were air-dried, passed through a 2-mm sieve, and finely ground. Primary and secondary carbonates present were removed using 1 M HCl prior to the stable C isotope-ratio analysis. A detailed description of sample preparation is available in Ref. [5].

## 2.2. Measurements of total organic carbon and $\delta^{13}\text{C}$ of soil

Soil samples were analysed for total C and stable C-isotope ratio using a Tracemass<sup>®</sup> isotope-ratio mass spectrometer (Europa Scientific, Crewe, UK) interfaced with a Roboprep unit. Ten- or 20-mg aliquots of soil (depending on the C concentration) were weighed into tin capsules, folded tightly to remove entrapped  $\text{CO}_2$ , combusted at 1,100°C and the resulting  $\text{CO}_2$  and  $\text{N}_2$  were passed through a reduction column in the Roboprep unit at 550°C, before detection by the mass spectrometer. Total organic C in soil samples was converted to  $\text{g m}^{-2}$  units based on soil sampling layer thickness and bulk density [5]. The  $\delta^{13}\text{C}$  of various parts of corn plants were measured along with alfalfa and bromegrass residues. One-mg samples of plant material were used for analysis and the mass spectrometer was operated as described above.

## 2.3. Estimation of corn-derived carbon ( $\text{C}_4\text{-C}$ )

The fraction of C originating from corn in the SOM ( $f_{\text{C}_4}$ ) was calculated using the following two end-member mixing model given in Ref. [5]:

$$f_{\text{C}_4} = \frac{(\delta^{13}\text{C}_{\text{SOM}} - \delta^{13}\text{C}_{\text{C}_3})}{(\delta^{13}\text{C}_{\text{Corn}} - \delta^{13}\text{C}_{\text{C}_3})} \quad (1)$$

where

$\delta^{13}\text{C}_{\text{SOM}}$  is the  $\delta^{13}\text{C}$  of soil from corn and corn-forage rotation plots,

$\delta^{13}\text{C}_{\text{Corn}}$  is the  $\delta^{13}\text{C}$  of corn plant materials ( $-12.0\text{‰}$ ),

$\delta^{13}\text{C}_{\text{C}_3}$  is the  $\delta^{13}\text{C}$  of soil from original/native vegetation.

The fraction of C originating from original/native  $\text{C}_3$  plants in SOM ( $f_{\text{C}_3}$ ) is equal to  $1 - f_{\text{C}_4}$ .

## 2.4. Estimation of forage-derived carbon

### 2.4.1. Subtraction method

The quantity of soil organic C derived from forages ( $Q_{\text{FR}}$ ) was calculated by subtracting the quantity of  $\text{C}_3\text{-C}$  in the continuous corn plots ( $Q_{\text{C}_3\text{-Corn}}$ ) from the quantity of  $\text{C}_3\text{-C}$  (original  $\text{C}_3\text{-C}$  plus forage  $\text{C}_3\text{-C}$ ) in the corn-forage plots ( $Q_{\text{C}_3\text{-CF}}$ ):

$$Q_{\text{FR}} = Q_{\text{C}_3\text{-CF}} - Q_{\text{C}_3\text{-Corn}} \quad (2)$$

The quantities of  $\text{C}_3\text{-C}$  in corn and corn-forage soils were obtained by estimating the quantity of corn derived C and subtracting it from the total organic C (TOC).

The major assumptions of this method are: (i) the decay values for native or original  $\text{C}_3\text{-C}$  under continuous corn and corn-forage rotation are similar, and (ii) the quantity of native/original  $\text{C}_3\text{-C}$  in corn-forage rotation plots was similar to that of continuous corn plots at the start of the field trial.

### 2.5.2. Two end-member mixing model method

This method is based on the principle that SOM of corn-forage rotation plots is a mixture of forage C ( $\text{C}_3$ ) and corn C ( $\text{C}_4$ ) plus original or native  $\text{C}_3\text{-C}$  present in the soil prior to the introduction of corn. Therefore, when a forage crop is introduced after continuous corn, the resulting SOM can be divided into two pools, forage and non-forage derived ( $\text{C}_4\text{-C}$  plus original  $\text{C}_3\text{-C}$ ).

The continuous corn plots in this situation are considered as the background or control (similar to a native soil in determining C<sub>4</sub>-C in soil). Therefore, the fraction of C originating from forage in SOM (*f<sub>FR</sub>*) is calculated from the following two end-member mixing model:

$$f_{FR} = \frac{(\delta^{13}C_{SOMCF} - \delta^{13}C_{CNT})}{(\delta^{13}C_{FORAGE} - \delta^{13}C_{CNT})} \quad (3)$$

where

$\delta^{13}C_{SOMCF}$  is the  $\delta^{13}C$  of soil from the corn-forage rotation,

$\delta^{13}C_{FORAGE}$  is the  $\delta^{13}C$  of forage plant material ( $-27.8\lambda$  for bromegrass and  $-28.4\lambda$  for alfalfa),

$\delta^{13}C_{CNT}$  is the  $\delta^{13}C$  of soil from continuous corn plots (control).

Equation (3), in concept, is similar to Eq. (2) used to estimate C<sub>4</sub>-C in soil. However, in its present form, it is not valid to estimate *f<sub>FR</sub>* as the  $\delta^{13}C$  of soil from continuous corn plots, and corn plant materials are vastly different. In Ref. [6] a model was proposed for situations in which a considerable difference exists between  $\delta^{13}C$  of control soil and plant materials. Therefore, Eq. (3) becomes:

$$f_{FR} = \frac{(\delta^{13}C_{SOMCF} - \delta^{13}C_{CNT})}{(\delta^{13}C_{FORAGE} - \delta^{13}C_{Corn})} \quad (4)$$

where,

$\delta^{13}C_{Corn}$  is the  $\delta^{13}C$  of corn plant materials ( $-12.0\lambda$ ).

The model proposed in Ref. [6] is applicable to the corn-forage rotation plots since the  $\delta^{13}C$  of soil and plant materials of continuous corn plots are widely different. The fraction of original/native C<sub>3</sub>-C in corn-forage rotation plots (*f<sub>C3-FR</sub>*) is equal to  $1 - f_{C4-FR}$ .

### 3. RESULTS AND DISCUSSION

In comparison with continuous corn plots, the rotation of alfalfa and bromegrass with corn did not significantly (statistical data are not shown in detail here) affect the levels of organic C in the soil (Table I). This lack of significance is likely due to high variability of soil-C levels. However, plots that had forages in the rotation generally had higher levels of organic C compared to plots of continuous corn.

TABLE I. EFFECT OF CORN FORAGE ROTATION ON TOTAL ORGANIC C IN SOIL

Soil depth (cm)	Continuous corn	Corn-bromegrass TOC (g m <sup>-2</sup> soil)	Corn-alfalfa
0 – 5	1,164 (125) <sup>a</sup>	1,487 (201)	1,180 (149)
5 – 10	1,271 (127.3)	1,116 (266)	1,171 (22.9)
10 – 15	1,239 (90.6)	1,325(113)	1,195 (108)
15 – 20	1,145 (216.1)	1,280 (133)	1,167 (92.2)
20 – 25	845 (302.8)	1,139 (414)	997 (416)
25 – 30	383 (75.2)	866 (485)	659 (312)
30 – 40	589 (154.4)	731 (225)	1,054 (1,045)
40 – 50	319 (23.2)	639 (336)	666 (435)
Total	6,954 (753.8)	8,584 (1,947)	8,087(2,060)NS <sup>b</sup>

<sup>a</sup>Standard deviation of the mean (n=4). <sup>b</sup>Not significant.



TABLE II.  $^{13}\text{C}$  NATURAL ABUNDANCE OF SOIL

Soil depth (cm)	Continuous corn	Corn-bromegrass $\delta^{13}\text{C}(\lambda)$	Corn-alfalfa	Forest
0 – 5	–22.6 (0.33) <sup>a</sup>	–24.7 (0.42)	–24.8 (0.52)	–26.7 (0.23)
5 – 10	–22.9 (0.55)	–24.3 (0.28)	–24.8 (0.43)	–26.6 (0.41)
10 – 15	–22.7 (0.32)	–24.2 (0.43)	–24.8 (0.48)	–26.5 (0.25)
15 – 20	–22.8 (0.27)	–24.1 (0.21)	–24.3 (0.38)	–26.7 (0.30)
20 – 25	–23.1 (0.41)	–24.3 (0.22)	–24.3 (0.55)	–26.9 (0.29)
25 – 30	–23.1 (0.44)	–24.2 (0.21)	–24.5 (1.04)	–27.1 (0.42)
30 – 40	–23.6 (0.17)	–23.8 (0.48)	–24.2 (1.40)	–27.4 (0.61)
40 – 50	–23.8 (0.46)	–23.8 (0.27)	–24.6 (0.66)	–27.5 (0.78)

<sup>a</sup>Standard deviation of the mean (n=4).

TABLE III. CORN DERIVED CARBON ( $\text{C}_4\text{-C}$ ) IN SOIL ORGANIC MATTER

Soil depth (cm)	Continuous corn	Corn-bromegrass $\text{C}_4\text{-C}$ ( $\text{g m}^{-2}$ soil)	Corn-alfalfa
0 – 5	318 (27.7) <sup>a</sup>	195 (0.05)	145 (31.6)
5 – 10	324 (75.1)	175 (21.5)	143 (36.1)
10 – 15	321 (30.5)	209 (42.1)	140 (44.3)
15 – 20	301 (67.4)	221 (28.7)	190 (31.8)
20 – 25	215 (86.0)	189 (58.5)	165 (53.4)
25 – 30	88.4 (16.2)	163 (84.5)	99.8 (31.1)
30 – 40	143 (35.1)	174 (72.3)	153 (61.2)
40 – 50	74.2 (7.8)	147 (63.8)	114 (49.1)
Total	1,785 (196)	1,475 (276)	1,151.6 (104)* <sup>b</sup>

<sup>a</sup>Standard deviation of the mean (n=4). <sup>b</sup>\*=Significant at  $P=0.05$ .

The introduction of forages markedly influenced the  $\delta^{13}\text{C}$  (Table II). Soil from continuous corn plots was more highly enriched in  $^{13}\text{C}$  than was that in the corn-forage rotation plots in the 0 to 25 cm zone. The  $\delta^{13}\text{C}$  values in the 25- to 50-cm depth were not significantly affected by cropping treatment.

The quantity of  $\text{C}_4\text{-C}$  was affected by rotating forages with corn (Table III). Forage plots had lower  $\text{C}_4\text{-C}$  quantities compared to continuous corn plots, except at lower depths. The total amount of  $\text{C}_4\text{-C}$  in the 0- to 50-cm layer was significantly affected by corn-forage rotation. With continuous corn, 26% of the C was derived from corn, significantly higher than in the corn-forage plots.

The continuous-corn plots sequestered  $61.5 \text{ g m}^{-2} \text{ yr}^{-1}$  of corn-derived C compared to 77.6 and 60.6  $\text{g m}^{-2} \text{ yr}^{-1}$  under 9 years of brome grass and 9 years of alfalfa, respectively. Brome grass appeared to be more effective in sequestering corn-derived C compared to alfalfa.

TABLE IV. FORAGE-DERIVED CARBON ( $C_F$ -C) IN SOIL ORGANIC MATTER AS ESTIMATED BY THE SUBTRACTION (SUB) AND TWO END-MEMBER MIXING MODEL (TEM) METHODS

Soil depth (cm)	Corn-bromegrass		Corn-alfalfa	
	SUB	TEM	SUB	TEM
	$C_F$ -C ( $g\ m^{-2}$ )			
0–5	447 (296) <sup>a</sup>	198 (81.4)	189 (253)	161 (55.9)
5–10	—	104 (80.9)	81.2 (72.0)	138 (31.2)
10–15	198 (97.3)	122 (33.5)	136 (169)	151 (58.2)
10–20	215 (210)	106 (28.4)	133 (218)	103 (24.8)
20–25	319 (357)	95.1 (52.1)	202 (539)	72.2 (47.1)
25–30	408 (425)	41.7 (54.5)	264 (330)	50.4 (76.8)
30–40	110 (229)	10.8 (13.8)	454 (886)	102 (176)
40–50	247 (262)	11.1 (13.9)	307 (404)	40.9 (61.2)
Total	1,940 (1,616)	689 (270)	1,767 (2,217)	819 (424)

<sup>a</sup>Standard deviation of the mean (n=4).

The estimates of forage-derived C were not in agreement for the two methods used (Table IV). The subtraction method, in which the quantity of  $C_3$ -C of continuous corn soil is used as the baseline to estimate the forage-derived C, estimated higher quantities of forage-derived C compared to the mixing model method based on  $\delta^{13}C$ .

The main assumption in both methods is that there is no differential decomposition and stabilization of soil C under monoculture compared with rotations. The study site had been cropped for several decades and dynamics of original or native  $C_3$ -C would not be affected by the present crop-management practices; labile fractions that are susceptible to management or cropping changes would have been lost within a few years of forest clearing. Therefore, the longer the period of transition from forest/native vegetation to cropland, the more likely the assumption is applicable.

Since the subtraction method is based on C quantities in soil, spatial variability of C can cause problems. For instance, some depths of corn-forage plots had lower total  $C_3$ -C than did continuous corn plots, resulting in negative values for forage-derived C. Therefore, the assumption of similar original or native  $C_3$ -C quantities at all depths was false.

Ref. [7] assumed that the quantity of C derived from prairie ( $C_4$ ) was similar in wheat- and manure-applied corn plots, in estimating the contribution from manure ( $C_3$ ) and corn ( $C_4$ ) to SOM in corn plots. Ref. [4] used an approach similar to the subtraction method to quantify legume-derived C in mixed communities of grasses ( $C_4$ ) and legumes ( $C_3$ ), with the assumption that the quantity of original  $C_3$ -C was equal in grass and grass-legume stands. The main difference between the method used in Ref. [4] and the subtraction method used in this study is that Ref. [4] estimated the quantity of legume-derived C using a model and the quantity of grass-derived C was estimated by difference. When used in the present study, the model in Ref. [4] estimated forage-derived C quantities similar (data not shown) to those estimated by the subtraction method. Ref. [4] stated that the drawback in the model is the dependency on total soil C measurements, which introduces larger variability than when estimates are based solely on  $\delta^{13}C$  signatures.

The method based on the two end-member mixing model estimated lower quantities of forage-derived C compared to the subtraction method. In this method, all estimations were based on  $\delta^{13}C$  of SOM of corn-forage and continuous corn plots. The  $\delta^{13}C$  of corn and forage residues were also used in the model. Ref. [3] used a similar approach to quantify legume ( $C_3$ ) and grass ( $C_4$ ) contribution to SOM in a mixed legume-tropical grass stands.

Estimations by the mixing model method of the forage-derived C remaining were lower than those with the subtraction method by 55 to 65%. Unlike the subtraction method, quantities of original C<sub>3</sub>-C in the soil of corn-forage plots were estimated by difference in the mixing model method. Continuous corn resulted in the lowest amounts of original C<sub>3</sub>-C in soil compared to corn-forage rotation plots; however, these differences were not statistically significant (data not shown).

It is possible that the isotopic mixing model underestimated the forage-derived C. However, examination of data in Ref. [3] suggested no over- or under-estimation by the mixing model method. For instance, the upper 0 to 2 cm of mixed grass-legume plots had about 28% more soil C (as a concentration) compared to grass plots, and this agreed with the observed 29% of legume-derived C in mixed grass-legume plots.

The other possibility to explain the observed differences in quantity of original C<sub>3</sub>-C among plots is that forage cropping may have a protective effect resulting in lower losses of original C<sub>3</sub>-C than under continuous corn. Therefore, estimation of original C<sub>3</sub>-C, as shown in the mixing model method, may yield information on effects (if any) of cropping on decay of original C<sub>3</sub>-C.

The observed differences in the estimates of forage-derived C, by the two methods, may be due largely to spatial variability of soil C at the study site. Of the two methods, the mixing model method appeared to be more robust. However, more research is required before selecting an approach. The results of the present study suggest that  $\delta^{13}\text{C}$  techniques can be used in mixed C<sub>3</sub>-C<sub>4</sub> systems to quantify contributing C inputs to SOM. With proper field layout and crop selection, the technique may be expanded to study SOM dynamics under a range of crop types and management conditions.

## REFERENCES

- [1] BALESIDENT, J., et al., Natural <sup>13</sup>C abundance as a tracer for the studies of soil organic matter dynamics, *Soil Biol. Biochem.* **19** (1987) 25–30.
- [2] HUGGINS, D.R., et al., “Carbon sequestration in corn-soybean agroecosystems”, *Soil Management and the Greenhouse Effect* (LAL, R., Ed.), Lewis Publishers, Boca Raton (1995) 61–68.
- [3] RAO, I.M., et al., The use of carbon isotope ratios to evaluate legume contribution to soil enhancement in tropical pastures, *Plant Soil* **162** (1994) 177–182.
- [4] CADISCH, G., GILLER, K.E., Estimating the contribution of legumes to soil organic matter build up in mixed communities of C<sub>3</sub>/C<sub>4</sub> plants, *Soil Biol. Biochem.* **28** (1996) 823–825.
- [5] WANNIARACHCHI, S.D., et al., Tillage effects on the dynamics of total and corn-residue-derived soil organic matter in two southern Ontario soils, *Can. J. Soil. Sci.* **79** (1999) 473–480.
- [6] BALESIDENT, J., MARIOTTI, A., “Measurement of soil organic matter turnover using <sup>13</sup>C natural abundance”, *Mass Spectrometry of Soil*. (BOUTTON, T.W, YAMASAKI, S., Eds.), Marcel Dekker, Inc., New York (1996) 83–111.
- [7] BALESIDENT, J., et al., Soil organic matter turnover in long-term field experiment as revealed by carbon-13 natural abundance, *Soil Sci. Soc. Am. J.* **52** (1988) 118–124.

## PRODUCTION OF LABELLED PLANT MATERIALS TO TRACE THE FATE OF RESIDUE-DERIVED CARBON, NITROGEN, AND SULPHUR

P. BASILIO-SANCHEZ, G. BLAIR, R. TILL, M. FAINT  
Agronomy and Soil Science, University of New England,  
Armidale, Australia

### Abstract

A technique to produce multiple-labelled ( $^{14}\text{C}$ ,  $^{15}\text{N}$ ,  $^{35}\text{S}$ , and  $^{13}\text{C}$ ,  $^{14}\text{C}$ ,  $^{15}\text{N}$ ) plant materials was developed to study the fate of residue-derived C, N and S following their incorporation into soil. *Flemingia* (*Flemingia macrophylla*), medic (*Medicago truncatula*), and rice (*Oryza sativa*) plants were grown in pots and labelled by adding  $^{15}\text{N}$  and  $^{35}\text{S}$  solutions to the potting mix every 4 days for 6 weeks. These pots were located in a labelling chamber (2.5×1.3×1 m), constructed from aluminium and PVC frames and covered with ethyl-vinyl alcohol film. Inside the chamber,  $^{14}\text{CO}_2$  and  $^{13}\text{CO}_2$  were generated by the reaction of  $\text{Na}_2^{14}\text{CO}_3$  or  $\text{Na}_2^{13}\text{CO}_3$  with lactic acid, and circulated by a commercial air-conditioning unit, set in recycling mode. The level of  $\text{CO}_2$  within the chamber was monitored using an infrared gas analyser; the concentration was allowed to drop from 350 to 300 ppm before the  $^{14}\text{CO}_2$  pulse was introduced. When the  $\text{CO}_2$  concentration further declined to approximately 180 ppm and became steady,  $^{12}\text{CO}_2$  was introduced into the chamber from a gas cylinder to return the concentration to 350 ppm. Sequential  $^{12}\text{CO}_2$  pulsing continued for the rest of the day or until 6:00 p.m. The frequency of labelling increased from once a week to four times a week as plant biomass increased. Labelled residues of medic and flemingia were incorporated into a sandy loam soil, and Japanese millet (*Echinochloa frumentacea*) was grown for 108 days in a glasshouse. The fate of the C, N, and S in the residues was traced in the plant, soil and leachate. Medic had a faster decomposition rate than did flemingia; almost half of its  $^{14}\text{C}$  disappeared by 41 days. The addition of medic hay resulted in significantly higher N and S contents in the tops of the first crop of millet, lower  $^{15}\text{N}$  and  $^{35}\text{S}$  recovery in soil, and higher  $^{15}\text{N}$  and  $^{35}\text{S}$  leaching losses. Flemingia leaves released a smaller proportion of  $^{15}\text{N}$  and  $^{35}\text{S}$ , which resulted in higher  $^{15}\text{N}$  and  $^{35}\text{S}$  recovery in soil and in the tops of the second crop of millet.

### 1. INTRODUCTION

Isotope-labelled plant materials have been widely used to study decomposition rates, nutrient-release patterns, and transformation in soil of plant-derived nutrients. Early studies on decomposition made use of  $^{14}\text{C}$ -labelled plant materials produced by growing plants in an atmosphere enriched with  $^{14}\text{CO}_2$  [1–3]. However, due to health and environmental hazards associated with radioactive materials, the use of the stable isotope  $^{13}\text{C}$  has become more popular. Numerous designs of C-isotope labelling chambers have been published, ranging from simple enclosures consisting of a polyethylene tent that can be punctured and sealed [4] to rigid chambers with sophisticated control equipment [5,6]. Plants can be continuously exposed to labelled  $\text{CO}_2$  or given a single pulse, depending on the researcher's objectives and resources.

Isotope-labelled plant materials have been useful in investigating cycling of plant-derived N in soil. Since the cycling of C and N are closely linked, dual-labelled plant materials— $^{14}\text{C}$ ,  $^{15}\text{N}$  [2,7] and  $^{13}\text{C}$ ,  $^{15}\text{N}$  [8]—are often produced.

This research was undertaken to produce triple-labelled ( $^{14}\text{C}$ ,  $^{15}\text{N}$ ,  $^{35}\text{S}$ , and  $^{13}\text{C}$ ,  $^{14}\text{C}$ ,  $^{15}\text{N}$ ) flemingia (*Flemingia macrophylla*), medic (*Medicago truncatula*), and rice (*Oryza sativa*) materials to trace the fate of residue-derived C, N, and S during decomposition. Incorporation of both stable and radioactive C isotopes into the plant tissue was also explored.

The use of labelled flemingia and medic is important in measuring turnover of crop residues in farming systems where prunings of leguminous shrubs/trees and legume crops are used as green manures. The labelled rice straw is particularly useful in studying decomposition in agricultural systems where straw is left on the soil surface or incorporated into the soil.

## 2. MATERIALS AND METHODS

### 2.1. Production of labelled plant materials

#### 2.1.1. Growth and maintenance of plants

Flemingia, medic and rice were grown in a 1:1 vermiculite:sand mixture in pots that were plastic-lined to prevent isotopic contamination of the surroundings. The pots were watered to field capacity by removing them from the chamber. Nutrient solution (Aquasol supplemented with  $\text{MgSO}_4$ ) was applied once per fortnight, then increased to weekly. Pesticides were applied when necessary.

Flemingia plants were grown to about 50 cm then heavily defoliated, and the medic and rice plants were severely pruned before labelling, to ensure that the newly formed biomass would have a high enrichment.

#### 2.1.2. Labelling chamber

The chamber (Fig. 1) was 2.5 m long, 1.3 m wide and 1 m high, large enough to produce sufficient quantities of plant material for the incubation studies. The frame was made from aluminum and PVC pipes and the sides from clear, gas-proof ethyl-vinyl alcohol film. The three sides of the chamber were permanently taped to the floor and sandbags were used to produce a permanent and gas-tight seal. The front was left unsealed to allow removal of plants for watering and application of  $^{15}\text{N}$  and  $^{35}\text{S}$  solution. The  $^{14}\text{CO}_2$  and  $^{13}\text{CO}_2$  were generated from the reaction of labelled  $\text{Na}_2\text{CO}_3$  with lactic acid injected through a thin plastic tube that ran through the side of the chamber.

A commercial air-conditioning unit was attached on one side to circulate the air and regulate the temperature to about  $25^\circ\text{C}$ . When cloudy and additional lighting was required, mercury vapour lamps located in the glasshouse above the chamber were turned on to maintain a daylength of 12 h. The  $\text{CO}_2$  concentration inside the chamber was monitored by an infrared gas analyser (ADC Type 225 Mk3).

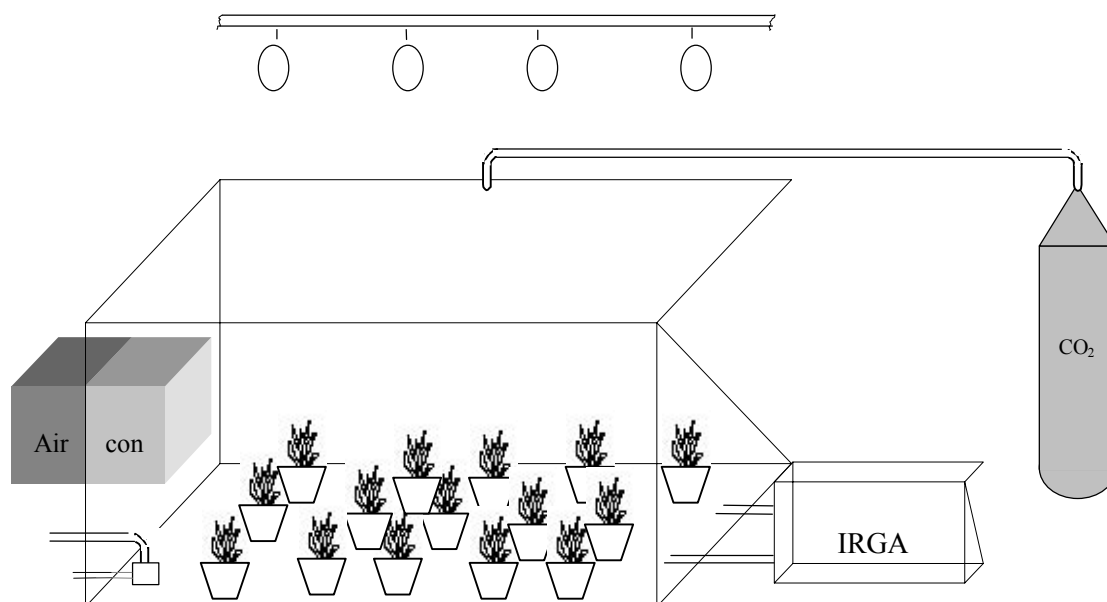


FIG. 1. Set-up of the labelling chamber.

#### 2.1.3. $^{14}\text{C}$ , $^{15}\text{N}$ , and $^{35}\text{S}$ labelling

The pulses of labelled  $\text{CO}_2$  were administered between 9:00 and 10:00 a.m. when photosynthetic activity was expected to be high. The  $\text{CO}_2$  concentration inside the chamber was allowed to drop from 350 to 300 ppm before the  $^{14}\text{CO}_2$  pulse was introduced. When the  $\text{CO}_2$  concentration declined to

approximately 180 ppm and became steady,  $^{12}\text{CO}_2$  was introduced from a gas cylinder to return the concentration to 350 ppm. Sequential  $^{12}\text{CO}_2$  pulsing continued for the rest of the day, or until 6:00 p.m. when artificial lighting was used, to maximize uptake of  $^{14}\text{CO}_2$ . The chamber was closed overnight to contain any  $^{14}\text{CO}_2$  released during respiration and to prevent  $^{14}\text{CO}_2$  leakage into the atmosphere. Additional pulses of  $^{12}\text{CO}_2$  were administered the next day before opening the chamber to expose the plants to natural conditions.

Although 2.5 MBq of labelled C was administered each time, the frequency of labelling increased from once a week to four times a week as plant biomass increased. The plants were pulse-labelled with  $^{14}\text{CO}_2$  fifteen times during the growing season, rotating the pots prior to each labelling to ensure uniformity of labelling.

Nitrogen-15 and  $^{35}\text{S}$  labelling was accomplished by adding 10 mL of solution containing 0.33 mg  $^{15}\text{N}/\text{mL}$  ( $^{15}\text{NH}_4\text{Cl}$  at 98.94 atom %) and 93 kBq  $^{35}\text{S}/\text{mL}$  every 4 days for 6 weeks to the surface of the pot using a syringe.

#### 2.1.4. $^{13}\text{C}$ , $^{14}\text{C}$ , and $^{15}\text{N}$ labelling

The technique for producing  $^{13}\text{C}$ ,  $^{14}\text{C}$ , and  $^{15}\text{N}$ -labelled plant materials was similar to that described above, except that  $\text{Na}_2^{13}\text{CO}_3$  was mixed with the  $\text{Na}_2^{14}\text{CO}_3$  solution before addition of lactic acid to liberate  $^{13}\text{CO}_2$  and  $^{14}\text{CO}_2$  inside the chamber.

The same flemingia plants grown for the first labelling were used while new medic and rice plants were established. The plants were again heavily pruned prior to labelling.

The amount of  $\text{Na}_2^{13}\text{CO}_3$  (99%) used for each pulse increased from 0.5 g in the first week to 1.0 g in the second and third weeks, then to 1.9 g for the succeeding weeks. It was reduced to 1.0 g when only the rice plants were left to mature. A constant pulse of 2.5 MBq  $^{14}\text{C}$  was administered throughout the labelling period.

Since the plants were not all harvested at the same time, they received varying amounts of C label: 21.6 g  $\text{Na}_2^{13}\text{CO}_3$  and 32.5 MBq  $^{14}\text{C}$  for medic, 33.0 g  $\text{Na}_2^{13}\text{CO}_3$  and 47.5 MBq  $^{14}\text{C}$  for flemingia, and 37.0 g  $\text{Na}_2^{13}\text{CO}_3$  and 57.5 MBq  $^{14}\text{C}$  for rice.

#### 2.1.5. Harvesting

The medic and rice plants were cut close to the surface of the potting medium, whereas the flemingia leaves were separated from the stems. The labelled plant material from all the pots of the same species was bulked before oven-drying at 50°C. Medic tops, rice straw, and flemingia leaves were cut into lengths of 2 to 3 cm, mixed to ensure uniformity, then stored for the glasshouse experiment.

### 2.2. Glasshouse experiment

The soil was a sandy loam collected from the upper 30 cm of an unfertilized native pasture near Uralla, NSW, Australia. Bulk soil was passed through a rotor to remove plant materials, air-dried, sieved to <2mm, and stored at room temperature prior to use. It contained 0.84% total C, 0.074% total N, and had a  $\delta^{13}\text{C}$  of -16.74‰.

Polyvinyl chloride (PVC) cylinders (30 cm long  $\times$  15 cm inner diameter) were divided into three 9-cm layers; each filled with 2.1 kg of soil, and referred to as top, middle, and bottom layers. The labelled plant materials were incorporated into the surface 5 cm of the top layer at a rate equivalent to 3 t/ha, 1 day after the application of basal macro- and micro-nutrients. Japanese millet (*Echinochloa frumentacea*) was sown at ten seeds/pot 2 days after residue application (DAA), and later thinned to six seedlings/pot.

Three replicates of each residue, including a control treatment with no residue, were laid out in a randomized complete block design. Japanese millet was pruned at 31 DAA and harvested 41 and 71 DAA. Another crop of millet was established and harvested after a further 37 days (108 DAA). The soil was destructively sampled at 41, 71, and 108 DAA. Leaching commenced at 31 DAA, then weekly thereafter for 11 weeks. Leachate was collected 1 day after watering each pot 25% above field capacity.

## 2.3. Analytical methods

### 2.3.1. Sample preparation

Samples of Japanese millet were oven-dried, ground to pass a 0.5-mm sieve, and stored in tight plastic jars for analyses of nutrient concentration and radioactivity. Soil samples, collected from each layer were placed in separate plastic bags, cleared of visible roots and partially undecomposed residues, mixed thoroughly, subsampled, air-dried, ground to  $\leq 0.5$  mm, and stored in plastic jars at room temperature.

### 2.3.2. Chemical analyses

Carbon-14 and  $^{35}\text{S}$  were measured by digesting the Japanese-millet material with soluene-350 (Packard) and counted in a Packard TRI-CARB Liquid Scintillation Analyzer, Model 2000CA, after addition of 17 mL of scintillation liquid consisting of p-terphenyl, POPOP, teric and toluene.

A quench curve, consisting of a series of spiked plant materials with varying amounts (5–25 mg) solubilized with 2 mL soluene-350, was prepared to correct for counting interferences due to Soluene-350 and to the colour resulting from the digestion.

Since the beta spectra of  $^{14}\text{C}$  and  $^{35}\text{S}$  and the region setting for  $^{14}\text{C}$  (0–156.48 kV) and  $^{35}\text{S}$  (0–167.47) overlapped, it was difficult to calculate the activities of the individual radionuclides using regional selection settings as described for dual-labelled  $^3\text{H}$  and  $^{14}\text{C}$  by Packard [9]. However, these two radionuclides vary greatly in their half-lives: 5,730 years for  $^{14}\text{C}$  and 87.4 days for  $^{35}\text{S}$  [9]. Therefore, the samples were then counted at different times and the decrease in total radioactivity was attributed to the decay of  $^{35}\text{S}$ . The following equations were used to calculate radioactivity of  $^{14}\text{C}$  and  $^{35}\text{S}$  using the counts obtained at two dates:

$$\text{At } t_1: \quad \text{total Bq} = \text{Bq } ^{14}\text{C} + \text{Bq } ^{35}\text{S} \quad (1)$$

$$\text{At } t_2: \quad \text{total Bq} = \text{Bq } ^{14}\text{C} + \lambda \text{ Bq } ^{35}\text{S} \quad (2)$$

where

$\lambda$  is the decay factor:

$$\lambda = e^{-0.693(t/t_{1/2})}$$

To compare the use of Soluene-350 with acid digestion,  $^{14}\text{C}$  in the plant materials was measured by digestion with chromium trioxide-acid mixture as described by Amato [10]. Radioactivity ( $^{14}\text{C}$ ) of the soil sample was also measured following acid digestion.

Extractable soil sulphate was measured based on the method developed by Blair et al. [11]. Three-mL aliquots of extract were taken for measurement of  $^{35}\text{S}$  activity.

Total C,  $^{13}\text{C}$ , total N, and  $^{15}\text{N}$  concentrations were determined using a Carlo-Erba NA 1500 elemental analyser coupled with a Europa Tracermass Isotope Ratio Mass Spectrophotometer.

## 2.4. Calculations and statistical analyses

The proportion of compartment (soil or plant) N derived from added crop residues (%Ndfr) was calculated using the equation [12]:

$$\%Ndfr = \frac{(\text{atom \% excess of compartment})}{(\text{atom \% excess of added residue})} \times 100$$

and the total amount of residue N (RDN) in the compartment was calculated as:

$$RDN = \frac{(\text{compartment total N})(\%Ndfr)}{100}$$

The proportion of residue  $^{14}\text{C}$  or  $^{35}\text{S}$  derived from crop residue was calculated using:

$$\text{Proportion of residue } ^{14}\text{C} = \frac{(\text{radioactivity of compartment})}{(\text{radioactivity of added residue})}$$

Data were subjected to analysis of variance using NEVA [13]. Separation of means was performed using Duncan's Multiple Range Test (DMRT) at  $P = 0.05$ .

## 3. RESULTS AND DISCUSSION

### 3.1. Production of labelled plant materials

A total of 155 g oven-dry  $^{14}\text{C}$ ,  $^{15}\text{N}$ ,  $^{35}\text{S}$ -labelled medic and 185 g of flemingia leaves were produced from the first labelling. Carbon-14 analyses revealed average specific activities of 38.3 kBq  $^{14}\text{C}$ /g C for flemingia and 39.6 kBq  $^{14}\text{C}$ /g C for medic (Table I).

Flemingia leaves assimilated 9.1% of the total  $^{14}\text{C}$  administered throughout the labelling period, while medic tops assimilated 7.5%. This  $^{14}\text{C}$  recovery is low compared to the 50 to 80% efficiency of  $^{14}\text{C}$  labelling techniques estimated by Voroney et al. (cited in Ref. [14]) because the  $^{14}\text{C}$  translocated to the other plant parts was not recovered. The amounts of  $^{14}\text{C}$  incorporated into plant tissues were similar for flemingia (18.5 kBq  $^{14}\text{C}$ /g) and medic (18.0 kBq  $^{14}\text{C}$ /g).

Medic tops were more highly enriched in  $^{15}\text{N}$  and  $^{35}\text{S}$  than flemingia (Table I). Higher dry-matter production by flemingia increased isotopic dilution, hence the lower  $^{15}\text{N}$  and  $^{35}\text{S}$  enrichments obtained.

The plant materials produced in the second set of labelling were less enriched in  $^{14}\text{C}$  and highly enriched with  $^{13}\text{C}$  (Table II). Plants discriminate against the heavier isotope ( $^{14}\text{CO}_2$ ) during photosynthesis when they are supplied with both  $^{13}\text{CO}_2$  and  $^{14}\text{CO}_2$  [15]. Rice straw contained the lowest  $^{13}\text{C}$  and  $^{14}\text{C}$  enrichments due to translocation of label to the grains, as below ground translocation of assimilated carbon was reduced (less than 5%) during the grain-filling stage [16]. Since the same flemingia plants that were used in the first labelling were re-exposed to  $^{14}\text{CO}_2$ , the flemingia leaves contained more  $^{14}\text{C}$  compared to medic tops and rice straw.

TABLE I. DRY-MATTER YIELD, NUTRIENT CONCENTRATION AND SPECIFIC ACTIVITY OF FLEMINGIA LEAVES AND MEDIC HAY AFTER 6 WEEKS EXPOSURE TO  $^{14}\text{CO}_2$ ,  $^{15}\text{N}$  AND  $^{35}\text{S}$

Plant material	Dry matter (g)	C (%)	Sp. act. (kBq $^{14}\text{C}$ /g C)	N (%)	$^{15}\text{N}$ (atom %)	S (%)	Sp. act. (MBq $^{35}\text{S}$ /g S)
Flemingia	184	48.2	38.3	3.34	1.66	0.200	27.8
Medic	154	45.4	39.6	4.45	2.53	0.350	38.6



TABLE II. CARBON AND NITROGEN CONCENTRATION AND ISOTOPIC ENRICHMENT OF FLEMINGIA, MEDIC, AND RICE STRAW AFTER LABELLING WITH  $^{13}\text{C}$ ,  $^{14}\text{C}$  AND  $^{15}\text{N}$

Plant material	C (%)	$\delta^{13}\text{C}$ (‰)	$^{13}\text{C}$ (atom %)	Sp. Act (kBq $^{14}\text{C}$ /g C)	N (%)	$^{15}\text{N}$ (atom %)
Flemingia	44.1	107	1.229	13.1	3.45	1.69
Medic	43.6	117	1.240	8.73	3.28	3.64
Rice	40.8	86.4	1.206	7.33	1.62	2.10

### 3.2. Fate of residue C, N and S

Addition of medic hay resulted in significantly higher residue N and S in the millet tops. At early stages of decomposition (31 DAA), 40%  $^{15}\text{N}$  recovery and 25%  $^{35}\text{S}$  recovery were obtained in the medic treatment compared to 9.5%  $^{15}\text{N}$  and 9.4%  $^{35}\text{S}$  recovery in the flemingia treatment. The higher concentration of N and S in the medic contributed to the higher amounts of N and S released during decomposition [17]. The reverse occurred in the second crop of millet, with flemingia leaves producing significantly higher uptake of N and S. The N and S released during the rapid decomposition of medic was immediately available for uptake whereas the slower breakdown of flemingia resulted in slower N and S release, and consequently in higher N recovery in the soil (Table III).

The amount of  $^{14}\text{C}$  recovered in the topsoil layer was higher in the flemingia treatment at all sampling times (Table III). The amounts recovered from both treatments declined with time as decomposition progressed. After 41 days, almost half of the residue  $^{14}\text{C}$  had been released, suggesting that medic contained relatively higher proportions of easily decomposable  $^{14}\text{C}$  compounds than did flemingia [7].

TABLE III. RESIDUE  $^{14}\text{C}$ ,  $^{15}\text{N}$  and  $^{35}\text{S}$  RECOVERED (% OF ADDED) IN EACH SOIL LAYER AND IN THE LEACHATE, AS AFFECTED BY RESIDUE AND SAMPLING TIME

Recovery	Layer	Days after addition					
		41		71		108	
		Flemingia	Medic	Flemingia	Medic	Flemingia	Medic
$^{14}\text{C}$	Top	76a <sup>a</sup>	56b	74a	42b	60a	39b
	Middle	1.6c	1.2c	1.0c	1.5c	1.5c	0.7c
	Bottom	0.4c	2.2c	0.8c	1.1c	0.7 c	1.4 c
$^{15}\text{N}$	Top	77a	55b	74a	53b	72a	51b
	Middle	0.5c	1.8c	0.7c	2.7c	2.7c	2.5c
	Bottom	0.2c	1.1c	0.9c	1.1c	1.3c	1.5c
	Leach	0.005	0.33	0.022	0.36	0.027	0.36
$^{35}\text{S}$	Top	7.65b	10a	4.0a	4.2a	4.7a	3.6a
	Middle	1.1c	1.3c	0.37b	0.52b	0.41b	0.27b
	Bottom	0.16c	0.45c	0.25b	0.12b	0.57b	0.24b
	Leach	0.050	0.65	0.080	0.69	0.10	0.70

<sup>a</sup>Within a sampling time and component, means followed by the same letter are not significantly different at  $P = 0.05$  by DMRT

About half of the  $^{15}\text{N}$  from the medic residue application remained in the top layer, while 71 to 77% was recovered from the application of flemingia (Table III). The amounts recovered in the top layer declined with time in both treatments, as N uptake increased. Nitrogen-15 recovery in the middle and bottom layers increased with time as N was leached. A smaller amount of  $^{15}\text{N}$  was lost through leaching from the flemingia treatment than from the medic.

Medic contributed a higher amount of extractable S in all the soil layers at 41 and 71 days (Table III). No significant difference in the amount recovered was recorded at day 108 although higher recoveries were obtained in the flemingia treatment. Higher leaching losses occurred with the medic treatment.

#### 4. CONCLUSIONS

The simple labelling tent used here was effective in producing the considerable quantity of multi-labelled plant residues required for the glasshouse study. The use of  $^{14}\text{C}$ ,  $^{15}\text{N}$  and  $^{35}\text{S}$  residues allowed the fate of these important plant components to be traced in the plant, soil, and leachate.

#### ACKNOWLEDGEMENTS

This work was funded by the Australian Centre for International Agricultural Research (ACIAR) while the senior author held an AusAID Overseas Postgraduate Scholarship. We are also grateful for the assistance of Dr. Anthony Whitbread, Mrs. Leanne Lisle, and Mrs. Judi Kenny.

#### REFERENCES

- [1] JENKINSON, D.S., Studies on the decomposition of plant material in soil –1. Losses of carbon from  $^{14}\text{C}$  labelled ryegrass incubated with soil in the field, *J. Soil Sci.* **16** (1965) 104–115.
- [2] SHIELDS, J.A., PAUL, E.A., Decomposition of  $^{14}\text{C}$ -labelled plant material under field conditions, *Can. J. Soil Sci.* **53** (1973) 297–306.
- [3] JENKINSON, D.S., AYANABA, A., Decomposition of carbon-14 labeled plant material under tropical conditions, *Soil Sci. Soc. Am. J.* **41** (1977) 912–915.
- [4] DAHLMAN, R.C., KUCERA, C.L., Tagging native grassland vegetation with carbon-14, *Ecol.* **49** (1968) 1199–1203.
- [5] JENKINSON, D.S., The production of ryegrass labelled with carbon-14, *Plant Soil* **13** (1960) 279–290.
- [6] WAREMBOURG, F.R., PAUL, E.A., The use of  $\text{C}^{14}\text{O}_2$  canopy techniques for measuring carbon transfer through the plant-soil system, *Plant Soil* **38** (1973), 331–345.
- [7] AMATO, M., et al., Decomposition of plant material in Australian soils. IV\* Decomposition in situ of  $^{14}\text{C}$ - and  $^{15}\text{N}$ -labelled legume and wheat materials in a range of southern Australian soils, *Aust. J. Soil Res.* **25** (1987) 95–105.
- [8] BROADBENT, F.E., NAKASHIMA, T., Mineralization of carbon and nitrogen in soil amended with carbon-13 and nitrogen-15 labeled plant material, *Soil Sci. Soc. Am. Proc.* **38** (1974) 313–315.
- [9] PACKARD INSTRUMENT CO., INC., Tri-Carb Operation Manual, Publication No. 169-3029 Packard Instrument Co., Inc., Meriden, CT (1985).
- [10] AMATO, M., Determination of carbon  $^{12}\text{C}$  and  $^{14}\text{C}$  in plant and soil, *Soil Biol. Biochem.* **15** (1983) 611–612.
- [11] BLAIR, G.J., et al., A sulfur soil test for pastures and crops, *Aust. J. Soil Res.* **29** (1991) 619–26.
- [12] VANLAUWE, B., et al., Soil organic matter dynamics after addition of nitrogen-15-labeled *Leucaena* and *Dactyladenia* residues, *Soil Sci. Soc. Am. J.* **62** (1998) 461–466.
- [13] BURR, E.J., NEVA User's Manual, University of New England Computer Center, Armidale, NSW, Australia (1982).
- [14] THOMPSON, R.B. Pulse-labelling a cover crop with  $^{13}\text{C}$  to follow its decomposition in soil under field conditions, *Plant Soil* **180** (1996), 49–55.

- [15] VAN NORMAN, R.W., BROWN, A.H., The relative rates of photosynthetic assimilation of isotopic forms of carbon dioxide, *Plant Physiol.* **27** (1952), 691–709.
- [16] KEITH, H., et al., Input of carbon to soil from wheat plants, *Soil Biol. Biochem.* **18** (1986) 445–449.
- [17] JANZEN, H.H., KUCEY, R.M.N., C, N, and S mineralization of crop residues as influenced by crop species and nutrient regime, *Plant Soil* **106** (1988) 35–41.

## NITROGEN DYNAMICS AND BALANCE IN A LOWLAND RICE CROPPING SYSTEM

S. PHONGPAN

Division of Agricultural Chemistry,  
Department of Agriculture,  
Bangkok, Thailand

A.R. MOSIER

Agricultural Research Service,  
United States Department of Agriculture,  
Fort Collins, Colorado, United States of America

### Abstract

Two field experiments were conducted during consecutive dry and wet seasons of 1997 in central Thailand to determine the effects of rice-residue management on the fate and use efficiency of urea-N broadcast to lowland rice (70 kg N ha<sup>-1</sup>). Ammonia (NH<sub>3</sub>) losses during the 11 days following urea application were 7, 12, and 8% of the applied N from no-residue, burned-residue, and residue-treated plots, respectively, whereas the cumulative percentage of N<sub>2</sub> + N<sub>2</sub>O emission from added urea corresponded to 10, 4.3, and nil, respectively. During the first crop, only about 9% of the fertilizer N applied was recovered in grain and straw. Appreciable N fertilizer was either lost from the soil-plant system (27–33%) or remained in the soil at the end of the growing season (50–53%). Residual fertilizer-N recovery in the second crop was only about 5% of what was initially applied.

### 1. INTRODUCTION

Traditionally, rice residue is either removed from the field prior to land preparation for the next crop, burned, or left and incorporated later into puddled soil.

Urea is the major N fertilizer used for lowland rice fields in tropical Asia, but it is not effectively utilized by rice because of substantial losses, particularly by ammonia (NH<sub>3</sub>) volatilization [1]. This economic and environmental concern has led to identification of alternative management practices that can reduce N losses and increase the use efficiency of urea fertilizer.

The objective of this field study was to determine whether integrated use of urea fertilizer with residue-management practices (no residue, burned residue, untreated residue) affects gaseous losses of N. We also wanted to observe the impact of residue management and response of rice to broadcast urea (i.e. N uptake and grain yield), to assess the <sup>15</sup>N balance after the harvest of the first rice crop, and to appraise residual effects of applied N on the succeeding rice crop.

### 2. MATERIALS AND METHODS

Two field experiments were conducted in a rice-fallow-rice cropping sequence during the consecutive dry and wet seasons of 1997, on a Phimai clay (Fluvic Tropaquept) at the Suphanburi Rice Research Centre located in the Central Plain of Thailand. The characteristics of the surface 0.15-m layer were: pH 5.8 (1:1 soil:water); total N, 1.6 g kg<sup>-1</sup>; organic matter, 17 g kg<sup>-1</sup>; and CEC, 23 cmol kg<sup>-1</sup>. The experiment consisted of six treatments that were replicated four times in a randomized completed block design under the following management: (1) all straw removed; (2) all straw left on the soil surface (3.75 Mg dry straw ha<sup>-1</sup> containing 21.7 kg N ha<sup>-1</sup>, C:N = 67:1); (3) all straw burned; (4) prilled urea (46% N) broadcast into the floodwater shortly after transplanting (70 kg N ha<sup>-1</sup>); (5) same as (4) but with (2); and (6) same as (4) but with (3). The straw, either burned or left on the soil surface, was incorporated with a rake into the surface soil layer (~0.05 m) 1 week before transplanting the rice seedlings. Treatments 4, 5 and 6 had a 1.2×1.2 m microplot, bounded by galvanized steel (0.3-m deep) inserted 0.15 m into the soil. Nitrogen-15-labelled urea (10.1 atom% excess) was broadcast into the microplots at the same rate and time as the unlabeled urea. All plots received P and K at rates of 24

and 33 kg ha<sup>-1</sup> as triple superphosphate (46% P<sub>2</sub>O<sub>5</sub>) and potassium chloride (60% K<sub>2</sub>O), respectively. Three to a hill of 21-day-old seedlings (var. Suphanburi 1) were hand-transplanted at a 20 × 20 cm spacing.

### 2.1. NH<sub>3</sub> loss

For 11 days after urea application, water temperatures were measured in situ in treatments 4, 5, and 6 every 2 h throughout the day. At the same times, water samples were collected from these plots and analysed for pH and ammoniacal-N. Ammonia loss was calculated by a bulk aerodynamic method using wind speeds measured at 0.8 m above the floodwater surface, floodwater ammoniacal-N, pH, and temperature data at each sampling [2].

### 2.2. (N<sub>2</sub> + N<sub>2</sub>O) flux

Inside the plots of treatments 1, 2, and 3, PVC tubes (0.5 m long × 0.2 m diameter) were driven into the soil to a depth of 0.25 m at 1 day before transplanting. On the same day, shortly after rice seedlings were transplanted, an equivalent of 70 kg N ha<sup>-1</sup> as 60.2 atom% <sup>15</sup>N-labelled urea was added to each PVC tube. Emissions of N<sub>2</sub> + N<sub>2</sub>O were sampled at 1- to 2-day intervals for 12 days after N application. Each cylinder was covered with a 0.2-m high, plastic closed chamber at 1730 h which was removed at 0730 h the next day, and gas samples were collected from each chamber immediately after closing the chamber and at the end of each sampling period. The gas samples were analysed for N<sub>2</sub> + N<sub>2</sub>O by isotope ratio mass spectrometry [3].

### 2.3. N-15 balance

At crop maturity, sixteen rice hills were harvested from each microplot and separated into grain and straw. Soil samples were collected with two 0.13×0.4-m sampling boxes from the centre four-hill area [4] and sectioned into layers of 0 to 0.05, 0.05 to 0.10, and 0.10 to 0.20 m. Roots were removed from the soil and washed. Finely ground plant material and soil samples were analysed for total N and <sup>15</sup>N in duplicate as described by [5], using an automated combustion isotope-ratio mass spectrometer.

The remaining soil from each layer collected at the end of the dry-season experiment was saved and returned, layer by layer, to the original site in the microplot. After the first rice crop was harvested, the field was then left fallow for about 70 days before establishing the residual-effect study. The effects of the N treatments applied to the first crop were appraised during the succeeding wet season in the same field using the same layout without addition of fertilizer N.

### 2.4. N<sub>2</sub>O flux

During the fallow period, PVC tubes were re-established within each microplot for the same treatments and methodology as in the dry season. Each week, gas samples were taken 4 h after installing the chamber at 10 a.m. and analysed for N<sub>2</sub>O by gas chromatography [6].

## 3. RESULTS AND DISCUSSION

### 3.1. NH<sub>3</sub> volatilization – dry season

Ammonia losses were low on day 1, increased to maximum values on days 2 and 4, and then decreased to negligible amounts on day 11 (Fig. 1). When volatilization of NH<sub>3</sub> reached its maximum on day 4, significantly more was lost from the burned-residue treatment than from the no-residue and with-residue treatments. The cumulative NH<sub>3</sub> losses during the 11 days following urea application were 4.65, 8.13, and 5.64 kg N ha<sup>-1</sup> from no-residue, burned-residue, and with-residue treated plots, which corresponded to 7, 12, and 8% of the applied N, respectively. These losses are comparable to others previously reported with the bulk aerodynamic method at the same location [7–9].

### 3.2. (N<sub>2</sub> + N<sub>2</sub>O) emission – dry season

Nitrogen gas plus N<sub>2</sub>O emissions were first detected 3 days after N application in all treatments (Fig. 2). In the no-residue treatment, the highest N<sub>2</sub> + N<sub>2</sub>O emission rate (387  $\mu\text{g N m}^{-2} \text{ h}^{-1}$ ) was recorded 7 days after urea application; it then dropped off to the lowest flux on day 10 before increasing again until day 12. In contrast, maximum N<sub>2</sub> + N<sub>2</sub>O emission (233  $\mu\text{g N m}^{-2} \text{ h}^{-1}$ ) from the burned residue occurred on day 3 and then decreased to the lowest rate on day 7 and fluctuated slightly thereafter until day 12. Emissions of N<sub>2</sub> + N<sub>2</sub>O from the residue treatment were not detected above 10  $\mu\text{g N m}^{-2} \text{ h}^{-1}$  over the 12-day period. From day 3 to 12 following urea addition, N<sub>2</sub> + N<sub>2</sub>O emissions averaged 290, 110, and less than 10  $\mu\text{g N m}^{-2} \text{ h}^{-1}$  from no-residue, burned-residue, and with-residue treated plots, respectively. Using the urea atom % enrichment and the equation outlined in Ref. [10] the cumulative percent of N<sub>2</sub> + N<sub>2</sub>O emission due to urea addition corresponded to 10, 4.3, and nil, respectively.

### 3.3. Distribution of fertilizer N in soil

Approximately 84% of the applied N recovered was in the top 0 to 0.05 m (Table I). The amounts of residue N in the top 0 to 0.05 m ranged from 41 to 46% of that applied, with no significant differences among treatments. Less than 4% of the <sup>15</sup>N was found in 0.05- to 0.10-m layer and 3 to 6% of the applied N was in the 0.10- to 0.20-m layer, the deepest sampled. Results also showed that considerable amounts of applied <sup>15</sup>N-labelled urea (50–53%) remained in the soil after harvest of the dry-season rice crop and was unaffected by residue treatment.

### 3.4. Recovery of <sup>15</sup>N by rice plants – dry season

Results from the <sup>15</sup>N microplots of the 1997 dry-season experiment are presented in Table II. At crop maturity, fertilizer N recovered by the rice plants (grain, straw and roots) did not show significant differences among residue treatments. Recovery of fertilizer N in the grain was low, only 9 to 11% of that applied. The amounts of <sup>15</sup>N recovered in the straw and roots were similar in all treatments, ranging from 7 to 11% and 0.05 to 0.21%, respectively.

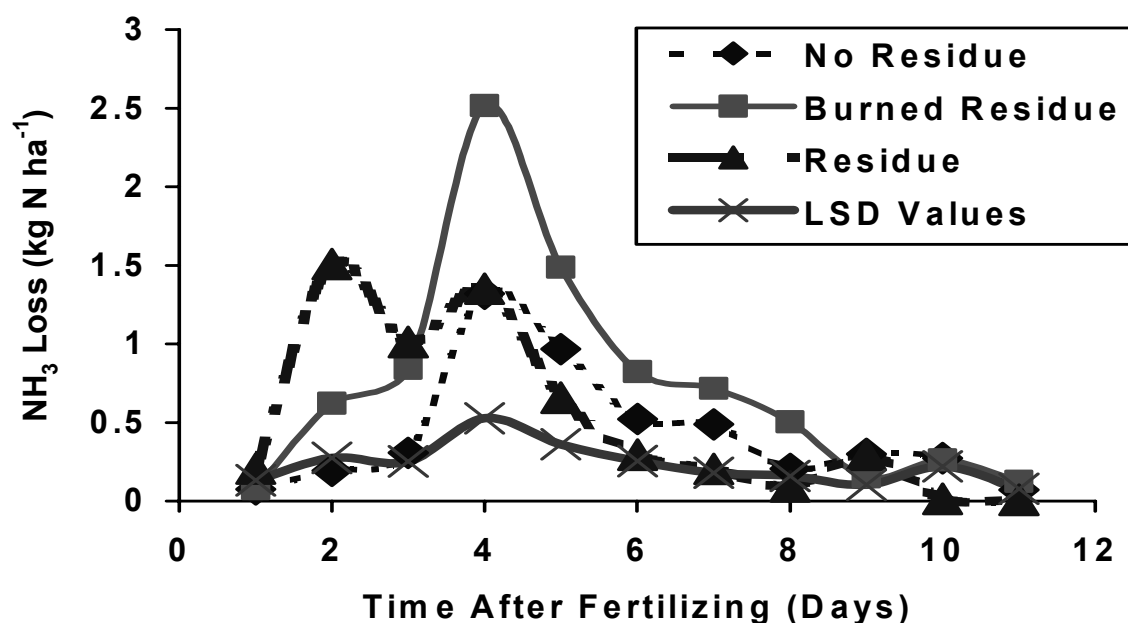


FIG. 1. Effect of residue treatment on NH<sub>3</sub> loss from flooded rice broadcast with urea

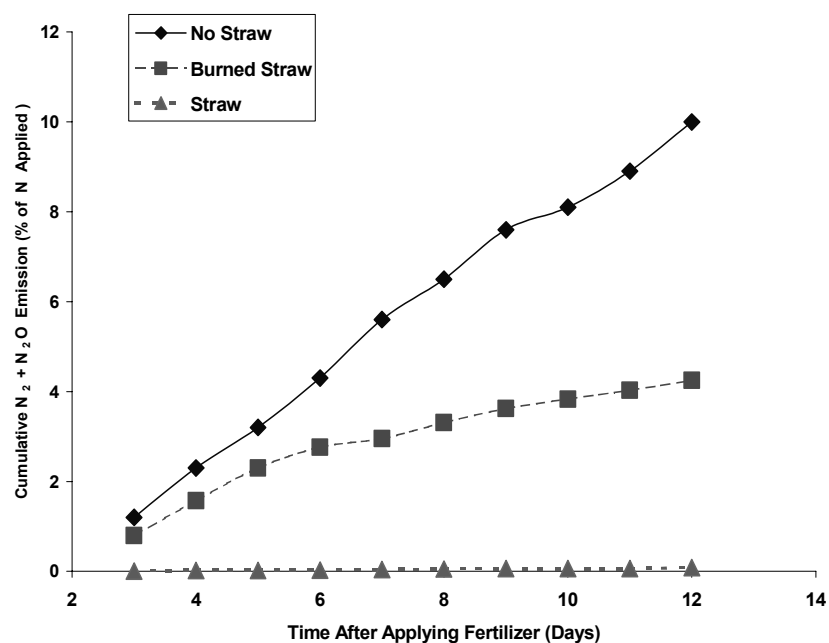


FIG. 2. Effect of residue treatment on dinitrogen plus nitrous oxide emission following urea fertilization of dry-season rice.

TABLE I. RECOVERY OF <sup>15</sup>N IN SOIL BY DEPTH (DRY SEASON)

Soil depth (m)	No residue	Burned residue	With residue	Significance of difference
	————	(% of applied <sup>15</sup> N)	————	
0–0.05	42	41	46	NS <sup>a</sup>
0.05–0.10	3.1	3.9	3.9	NS
0.10–0.20	6.1	5.0	2.7	NS
Total	51	50	53	NS

<sup>a</sup>Not significant at  $P > 0.05$ .

TABLE II. BALANCE OF <sup>15</sup>N IN THE SOIL-PLANT SYSTEM (DRY SEASON)

Treatment	<sup>15</sup> N recovery (% of N applied)				Unaccounted for
	Grain	Straw	Roots	Soil	
No residue	11a	11a	0.21a	51a	27a
Burned residue	9.4a	7.5a	0.17a	50a	33a
With residue	8.9a	8.8a	0.15a	53a	29a

Values in a column followed by the same letter are not significantly different at  $P > 0.05$  by DMRT.

TABLE III. UPTAKE OF RESIDUAL  $^{15}\text{N}$  BY THE SECOND RICE CROP AS AFFECTED BY PRIOR RESIDUE TREATMENT (WET SEASON)

Treatment	$^{15}\text{N}$ uptake			Recovery of $^{15}\text{N}$			
	Grain	Straw	Roots	Grain	Straw	Roots	Total
	———— (kg N/ha) ————			———— (%) ————			
No residue	1.67	1.28	0.02	2.4a	1.8a	0.03a	4.2
Burned residue	1.84	1.88	0.03	2.6a	2.7a	0.04a	5.3
With residue	1.90	1.53	0.03	2.7a	2.2a	0.04a	4.9

Values in a column followed the same letter are not significantly different at  $P > 0.05$  by DMRT.

Total recovery (18%) from residue-treated plots was slightly lower than that with the no-residue treatment (23%), and total  $^{15}\text{N}$  recovered (17%) was lowest when urea was broadcast on the burned-residue plots (Table II). This range of recovery was even lower than values reported at harvest for other lowland rice systems, i.e. in the range 30 to 50% [11–13]. However, results from a study conducted in an area adjacent to the present site, had recoveries of  $^{15}\text{N}$  from urea in above-ground rice biomass closer to 20% [7].

### 3.5. $^{15}\text{N}$ losses – dry season

The quantities of unaccounted-for  $^{15}\text{N}$ , presumed to have been lost as gaseous  $\text{N}_2$  emissions, ranged from 27 to 33% of the applied N, and were unaffected by residue treatments (Table II). These results are similar to those from previous field experiments [11,12] in which 20 to 28% of the applied N was lost during the crop-growing period following a basal incorporation of urea at transplanting.

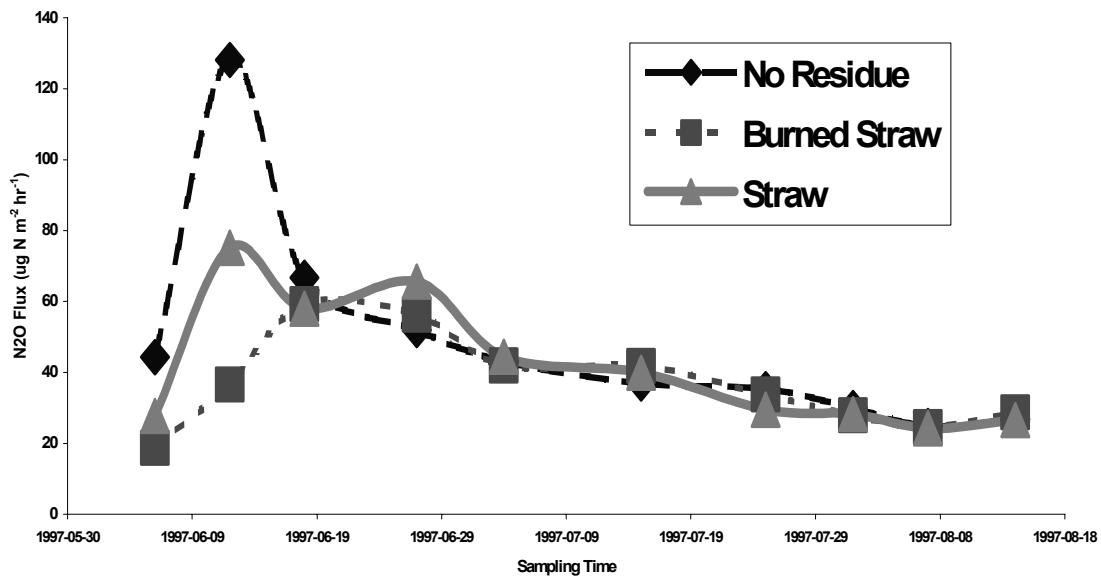


FIG. 3. Nitrous oxide emissions during the fallow period from dry-season rice plots without amendment, with burned straw or with rice-straw residue.

### 3.6. Nitrous oxide flux – during the fallow period between the dry and wet seasons

Higher amounts of  $\text{N}_2\text{O}$  were emitted from the no-residue plots than from the others (Fig. 3) in the first week of the fallow period [(6 June to 14 August) prior to flooding the soil for wet-season rice] and declined thereafter through the middle of August. On the whole, there were no significant differences in  $\text{N}_2\text{O}$  among treatments. Measurements at weekly intervals averaged 49, 37, and 42  $\mu\text{g N}$



$\text{m}^{-2} \text{h}^{-1}$ , and ranged from 25 to 128, 19 to 59, and 24 to 75  $\mu\text{g N m}^{-2} \text{h}^{-1}$  from no-residue, burned-residue, and with-residue treated plots, respectively.

### 3.7 Plant uptake of residual fertilizer N – wet season

Residue treatment did not appear to affect the uptake of residual N by the wet-season rice crop (Table III). When expressed in terms of a percentage of the  $^{15}\text{N}$  initially applied, no significant differences in fertilizer  $^{15}\text{N}$  recovery (grain, straw, and roots) were observed among treatments. Only 4 to 5% of the  $^{15}\text{N}$  applied to the dry-season crop was taken up by the succeeding rice. Low recoveries of residual labelled N in lowland rice soils by a subsequent crop have been reported before [e.g. 4,14].

### 3.8 Grain yield and N uptake

We found that, in the dry season, grain yield from the with-residue treatment was not significantly different from those with no-residue and burned-residue treatments (Table IV). Grain yield as well as N uptake for grain (but not for straw) were significantly increased on all N-treated plots compared with the residue treatments without N. In terms of grain yield and N uptake by rice, no long-term effect of residue treatment or of N applied to rice in the dry season was seen on succeeding rice in the wet season, indicating that considerable amounts of residual N in the soil after harvest were very slowly available and/or were less effective sources to meet the N requirement of the rice crop in the second season.

TABLE IV. MEAN GRAIN YIELD AND N UPTAKE OF RICE AT MATURITY AS AFFECTED BY UREA AND CROP-RESIDUE MANAGEMENT PRACTICES (DRY AND WET SEASONS)

Treatment	Dry season			Wet season		
	Grain yield (Mg/ha)	N uptake (kg N ha <sup>-1</sup> )		Grain yield (Mg/ha)	N uptake (kg N ha <sup>-1</sup> )	
		Grain	Straw		Grain	Straw
1. Straw removed	3.7c	35.6b	35.0b	4.0a	46.6a	65.9a
2. Straw left	4.0bc	39.7ab	36.9b	4.1a	41.9ab	45.3b
3. Straw burned	3.7c	38.4b	41.3ab	3.9a	45.4a	54.2ab
4. Urea broadcast	4.7a	46.2a	43.7a	3.8a	35.0b	45.3b
5. = 4 + 2	4.8a	48.5a	39.7b	3.9a	37.9ab	47.4b
6. = 4 + 3	4.2a	47.4a	41.1ab	3.7a	36.9b	53.1ab
CV (%)	6.5	12.3	16.2	9.1	12.4	19.5

Values in a column followed by the same letter are not significantly different at  $P > 0.05$  by DMRT.

### REFERENCES

- [1] DE DATTA, S.K., BURESH, R.J., Integrated nitrogen management in irrigated rice, *Adv. Soil Sci.* **10** (1989) 143–169.
- [2] FRENEY, J.R., et al., Estimating ammonia volatilization from flooded rice fields by simplified techniques, *Soil Sci. Soc. Am. J.* **49** (1985) 1049–1054.
- [3] MOSIER, A.R., et al., Evolution of dinitrogen and nitrous oxide from the soil to the atmosphere through rice plants, *Biol. Fertil. Soils.* **9** (1990) 61–67.
- [4] CAO, Z.H., et al., Nitrogen-15 balance and residual effects of urea-N in wetland rice fields as affected by deep placement techniques, *Soil Sci. Soc. Am. J.* **48** (1984) 203–208.
- [5] PORTER, L.K., MOSIER, A.R.,  $^{15}\text{N}$  Techniques and analytical procedures : Indo/U.S. Science and Technology Initiative U.S. Department of Agriculture, Agricultural Research Service, **ARS-95** (1992). 1–26.

- [6] MOSIER, A.R., MACK, I., Gas chromatographic system for precise, rapid analysis of N<sub>2</sub>O, Soil Sci. Soc. Am. J. **44** (1980) 1121–1123.
- [7] FRENEY, J.R., et al., Effect of urease, nitrification and algal inhibitors on ammonia loss and grain yield of flooded rice in Thailand, Fert. Res. **40** (1995) 225–233.
- [8] PHONGPAN, S., et al., Use of phenylphosphorodiamidate and N-(n-butyl) thiophosphorictriamide to reduce ammonia loss and increase grain yield following application of urea to flooded rice, Fert. Res. **41** (1995) 59–66.
- [9] CHAIWANAKUPT, P., et al., Use of urease, algal inhibitors and nitrification inhibitors to reduce nitrogen loss and increase the grain yield of flooded rice (*Oryza sativa* L.), Biol. Fertil. Soils **22** (1996) 89-95.
- [10] MOSIER, A.R., et al., Determination of dinitrogen emission and retention in floodwater and porewater of a lowland rice field fertilized with <sup>15</sup>N urea, Fert. Res. **19** (1989) 127–135.
- [11] SNITWONGSE, P., et al., Fate of nitrogen fertilizer applied to lowland rice on a Sulfic Tropaquept, Fert. Res. **16** (1988) 227–240.
- [12] SNITWONGSE, P., et al., Fate and efficiency of fertilizer nitrogen (<sup>15</sup>N labeled) in wetland rice soil in Thailand, Thai. J. Agric. Sci. **21** (1988) 277–283.
- [13] SATRUSAJANG, A., et al., Nitrogen-15 and sulfur 35 balances for fertilizers applied to transplanted rainfed lowland rice, Fert. Res. **28** (1991) 55–65.
- [14] PANDA, M.M., et al., Nitrogen utilization by lowland rice as affected by fertilization with urea and green manure, Fert. Res. **40** (1995) 215–223.



**SOIL AND WATER  
MANAGEMENT AND CONSERVATION**

(Session 3)



## EXPLOITS AND ENDEAVOURS IN SOIL WATER MANAGEMENT AND CONSERVATION USING NUCLEAR TECHNIQUES\*

S.R EVETT\*\*

Conservation and Production Research Laboratory,  
USDA-Agricultural Research Service,  
Bushland, Texas, United States of America

### Abstract

Soil water management and conservation efforts have been immensely aided by the advent of nuclear techniques since the 1950s, most notably by the introduction of the neutron moisture meter (NMM). At the end of World War II, the understanding of nuclear physics had increased tremendously, and there was a concerted effort to turn this knowledge to productive and peaceful uses. In 1949, a method for “the measurement of the moisture content of soil by the slowing of neutrons” was described and subsequent research and development resulted in commercial availability of the NMM by the late 1950s. Research on water management and conservation began replacing gravimetric sampling with the NMM almost immediately, and crop water use data for a wide range of annual and perennial crops in a wide range of soils and climates became available in the 1960s. Although weighing lysimeters remained an important tool for crop water use determination, the wide range of soil and climate effects could not have been investigated without the NMM due to the expense of lysimeters. Advantages of the method included, and still include, a relatively large sampling volume, so that fewer samples are required, and precision better than  $0.01 \text{ m}^3 \text{ m}^{-3}$  when correctly calibrated, which exceeds that of gravimetric sampling for equal numbers of samples. The NMM was used for irrigation scheduling in research in the 1950s. But, in the early 1970s, improvements in portability, reliability, and user interface allowed the NMM to become an important tool for irrigation scheduling in production farming. Many of the uses of the NMM require measurement of the change over time of soil water stored within and below a crop or plant stand root zone. Accurate measurement of changes in water content depends on the accuracy of the calibration equation slope. Calibrations are specific to soils and soil horizons, so site-specific calibration is required. Because errors in calibration slope are likely without correct calibration methods, research on calibration methods is discussed and guidelines for accurate calibration are given.

### 1. INTRODUCTION

At the end of World War II, the understanding of nuclear physics had increased tremendously, in particular the interactions of neutrons with atoms, which knowledge was essential to the development of the atomic bomb and fission reactors. There was a concerted effort to turn this knowledge to productive and peaceful uses. In 1949, George Pieper described a method for “the measurement of the moisture content of soil by the slowing of neutrons” [1]. In 1950, the US Civil Aeronautics Administration published Technical Report 127, describing a method for measuring soil moisture based on neutron thermalization [2]. Independently, Wilford Gardner and Don Kirkham developed essentially the same method, which was published in Soil Science [3]. The method is based on two facts. First, of the elements common in soils, hydrogen is by far the most effective in converting fast neutrons to thermal neutrons through collisions. Second, of the hydrogen-bearing soil constituents, water is usually the most plentiful and the only one that changes rapidly to an important extent.

The method uses a radioactive source of fast neutrons (mean energy of 5 MeV) and a detector of slow neutrons ( $\sim 0.025 \text{ eV}$  or 300 K). High-energy neutrons emitted from the source ( $\sim 10^{27}/\text{s}$ ) are either slowed through repeated collisions with the nuclei of atoms in the soil (scattering), or are absorbed by those nuclei. A small fraction will be deflected back to the detector. Of these, an even smaller fraction ( $\sim 10^3/\text{s}$ ) will have been slowed to thermal (room temperature) energy levels and will be detected. The most common atoms in soil (aluminium, silicon and oxygen) scatter neutrons with little energy loss

---

\*This work was prepared as part of a USDA employee's official duties and cannot legally be copyrighted. The fact that the publication in which the article appears is itself copyrighted does not affect the material of the U.S. Government, which can be reproduced by the public at will.

\*\*<http://www.cprl.ars.usda.gov/programs/>.

because they have much greater mass than a neutron. However, if the neutron collides with a hydrogen nucleus its energy is reduced on average to about half, because the mass of the hydrogen nucleus is the same as that of the neutron. On average, nineteen collisions with hydrogen are required to thermalize a neutron. Carbon, nitrogen, and oxygen are relatively less efficient as neutron thermalizers (about 120, 140 and 150 collisions, respectively). The concentration of thermal neutrons changes mainly with the hydrogen content of the surrounding material. On the time scales of normal interest in water management, changes in H and O content occur mainly due to changes in soil water content. Thus, the concentration of thermal neutrons can be directly related to soil volumetric water content.

The US Corps of Engineers contracted with Nuclear-Chicago Corporation<sup>1</sup> in 1955 to design and manufacture a portable field system; and by 1960 several hundred neutron moisture meters (NMMs) were in use [4]. They were produced by other companies and institutions around the world, and began to be used in agricultural and hydrological research. Early NMMs were two-piece units consisting of a scalar readout and associated counting circuitry in one enclosure, connected by a cable to a cylindrical probe that was locked in a radiation shield when not in use, and that was lowered into a cylindrical, cased hole in the soil for readings. The probe contained a radioactive source of fast neutrons and a detector tube for detecting thermalized neutrons. This system is a profiling NMM and has been used to measure soil profile water contents to many meters depth. Alternatively, the scalar and cable were connected to a flat-bottomed unit that contained both the source and detector tubes, and which was placed on the soil surface. In either case, precision of early units was lower than we see today. For instance, Hauser [5] analysed multiple soil profile readings and found that moisture measurements in 183-cm deep profiles would have to differ by more than 1.6 cm before being considered significantly different. He attributed about half the error to the imprecise timers available. Work through the 1950s and early 1960s focused on equipment designs to improve depth resolution, linearity of the calibration, and portability; and on laboratory and field tests to measure the volume of influence and its center in relation to the probe geometry.

For profiling NMMs, the measurement volume is approximately a sphere with radius of about 0.15 m in a wet clay soil and increasing to 0.5 m as water content declines to  $0.02 \text{ m}^3 \text{ m}^{-3}$  [6]. For a soil of specified volumetric water content ( $\theta_v$ ,  $\text{m}^3 \text{ m}^{-3}$ ), about 95% of the measured slow neutrons are from a sphere of radius  $R$  (cm) [7]:

$$R = 100/(1.4 + 10\theta_v) \quad (1)$$

However, since 1965 the design, detector efficiency, and source strength of profiling NMMs has changed such that measurement volumes may be smaller and depth intervals between readings as small as 0.15 m are useful [8].

For non-intrusive measurements, the surface NMM is placed on the soil surface. The source and detector are located just above the base of the meter and are commonly separated by several cm. The volume measured is roughly hemispherical and extends into the soil for a distance that decreases as the soil water content and density increase, and which varies from  $\sim 0.15$  m in wet soil to  $\sim 0.3$  m in dry soil [6]. The precision is less than can be attained with a profiling meter; and it suffers even more when soil moisture changes greatly with depth near the surface [9], a common occurrence. Good precision has been reported under fairly stringent conditions including: 1) flattening the surface to fit the gauge bottom with no air gaps, 2) marking the measurement site so that the gauge can be repeatedly placed in identical position, and 3) using a cadmium neutron absorber shield around the meter (except for the bottom) to reduce effects of surrounding vegetation [10]. However, even in the latter study the strong depth dependency of calibration coefficients and the inability to accurately estimate the depth of reading led to great uncertainty as to the accuracy of measurements. For example, calibrations developed for the surface to 2-cm and surface to 8-cm depth ranges could not be used to accurately determine the water content in the 2- to 8-cm depth range.

---

<sup>1</sup>Mention of trade names or other proprietary information is made for the convenience of the reader and does not imply endorsement, recommendation or exclusion by the USDA-Agricultural Research Service.

Extensive work measuring crop water use with the neutron probe began in the early 1960s in Israel [11,12] and in the late 1950s in the United States [13–15]. In Israel, water requirements were measured for eight field crops, five orchard crops, and four other species over a 20-year span from 1954 to 1974, with the NMM being used after 1963 [11,12]. In Israel, this work clearly demonstrated that the yield vs. water use relationship was not independent of climate. In the Southwestern United States, crop water requirements were measured for seventeen field crops using the NMM in the 1960s and 1970s [13, 14]. Work begun in California in the late 1950s found that the variability between sites in water use determinations by the soil water balance method was less when the NMM was used than when gravimetric methods were used [15].

Studies of irrigation efficiency necessarily include estimation of crop water use and determination of soil water distribution in the field. The NMM became an important tool for such investigations despite the difficulty of accounting for deep percolation losses or upward movement of water from shallow water tables [16]. One method of accounting for such vertical water fluxes is to use tensiometers placed at several depths in order to find two depths at which the soil water potential is so nearly equal that the driving force for water flux between those depths is negligible. The plane between these two depths is called the zero flux plane. The crop water use can then be calculated from the soil water balance for the zone from the surface to the zero flux plane [17].

Weighing lysimeters have been used for many years for precise (e.g., 0.05 mm) measurement of evaporation (E) and evapotranspiration (ET) from bare and cropped soils [18]. However, lysimeter installations suffer from some serious drawbacks including disturbance of the soil profile, interruption of deep percolation and horizontal flow components and uneven management of lysimeter compared to field soil [19]. Other drawbacks include heat flux distortions caused by highly conductive steel walls [20,21] and high cost, e.g., US\$ 65,000 [22] and US\$ 80,000 [23].

Alternatives to lysimetry for the measurement of E and ET (both mm) include mass balance techniques that involve measuring the components of the water balance equation for a soil profile of given depth:

$$\Delta S = P - (E \text{ or } ET) - D - R \quad (2)$$

where

$\Delta S$  is the change in soil profile water storage,  
P is precipitation (including irrigation),  
R is runoff, and  
D is deep percolation, i.e., water moving across the bottom boundary of the soil profile (all in mm).

Solving for E or ET gives

$$E \text{ or } ET = -\Delta S + P - D - R \quad (3)$$

Measurement intervals commonly range between hours and weeks and are usually no smaller than the required period of ET measurement. Measurement of each variable in the right-hand side of Eq. 3 presents its own unique problems, and it should be stated that lysimetry has three sources of measurement error as well [lysimeter mass ( $\Delta S$ ), precipitation (P), and runoff (R)]. However, the water balance technique is applicable in many situations for which lysimetry is inappropriate or impossible and is, in addition, much less expensive.

Soil profile water content measurement techniques range from destructive sampling using augers or coring tubes to non-destructive techniques such as gamma-ray attenuation, neutron thermalization and capacitance measurements in access tubes. Techniques also include various sensors including resistance blocks, heat flux based sensors, and time domain reflectometry (TDR) probes that are buried at specific depths. Destructive techniques are commonly avoided because they cannot repeatedly measure the same locations and the time involved in handling the samples. Of the non-destructive techniques, neutron thermalization (NS) was proposed by Van Bavel and Stirk in 1967 [24]



for ET studies and has often been used since [25,26]. Due to the small changes in water content associated with single day ET and the limited precision of NS, especially near the surface, the water balance method has usually been restricted to measurement of ET over periods of several days [27]. Wright [26] compared ET measured by a weighing lysimeter to that measured by soil water balance using NS and concluded that large errors in the water balance method occurred if the depth of the profile measured by NS did not exceed the depth of wetting due to irrigation. The errors were then due to excessive water flux through the bottom of the profile. Bertuzzi et al. [28] simulated sampling strategies that combined the NMM for measurements at 0.2 m and deeper with accurate thin layer sampling in the top 0.2 m of soil. They concluded that daily water use could be accurate to  $\pm 0.5$  mm with this method if the zero flux plane did not move below the bottom of the NMM access tubes or if accurate estimation of the flux rate could be obtained. They suggested that capacitance probes might be used for the accurate thin layer measurements. Evett et al. [29] investigated the joint use of TDR and NS for estimating ET and compared it to weighing lysimeter measurements with good results.

Early NMMs were not suitable for routine irrigation scheduling due to their bulk, weight, and difficult operation and data recording, and non-robust design [30]. Improvements in design addressed many of these concerns; and in the 1970s irrigation scheduling using the NMM became common [31]. Before the NMM, gravimetric sampling was the most common soil water measurement method for irrigation scheduling. The NMM proved to require less labour both because fewer samples were required and because the wait for soil to dry was eliminated, although it cost more to acquire the equipment [32]. Overall, of soil water monitoring methods available in the 1970s and 1980s, it had the “best combination of features for irrigation scheduling” [32]. Invention of a scheduling method that worked by tracking the rate of soil water depletion rather than depending on absolute water contents allowed the number of samples needed to be reduced to one or two tubes per uniformly irrigated field [31,32]. Comparison of the soil water tracking method with computerized scheduling based on estimates of crop water use showed that scheduling was more accurate with the tracking method, particularly over longer periods when computerized methods failed to account well for the status of soil water [31].

A recent search for research that used the NMM turned up over 1,100 papers published since 1970 that mentioned the NMM in the abstract. Certainly, many more research papers have been published that described the NMM as a routine and reliable method for soil water content measurement.

The NMM has influenced many important areas of investigation including:

- crop water use determination,
- irrigation efficiency determination,
- irrigation scheduling,
- tillage effects on crop water use,
- root water uptake patterns/soil effects,
- partitioning of rainfall and irrigation to runoff and infiltration,
- temporal and spatial variability of soil water content,
- measurement of soil hydraulic conductivity,
- wetting front movement studies,
- species and cultivar adaptation to water deficit stress,

to name relatively few.

It would be difficult to overestimate the importance of the neutron thermalization method in soil science and hydrological research and development over the past 50 years. It was the first useful, non-destructive method of repeatedly sampling the moisture content of soil profiles throughout and below the root zone. It led to the widespread measurement of crop water use values that are essential to irrigation management and the planning of large-scale irrigation developments. In 1998, a panel of scientists, expert in soil water measurement using time domain reflectometry (TDR), capacitance, and neutron thermalization methods, convened by the International Atomic Energy Agency, recommended that the neutron thermalization method not be replaced in the agency’s research and training programs [33]. Three reasons were given: i) the method measures a relatively large volume of soil compared

with TDR and capacitance instruments and so integrates across small-scale variability of soil properties and reduces the number of measurements needed, as well as reduces the sensitivity of the method to soil disturbance caused by installation, ii) the method is reliable and easy to use compared with others, and iii) the technology is mature, bringing to bear a large knowledge base of proven solutions to particular problems of use. To this I would add that the large volume of measurement makes field calibration much easier than it is for TDR and capacitance probes.

Of less practical importance, but still a valuable research tool, the gamma-ray attenuation method has been widely used for studies of soil bulk density and water content [34]. Before the introduction of TDR, the gamma-ray method was the best and practically the only way to obtain water content data for thin layers of soil. Many column studies have been done using the method, but field applications are relatively infrequent, except in soils engineering where the moisture/density gauge is commonly used to assess compaction of fill materials. Other important uses of nuclear techniques include the use of radioactive fallout products from nuclear bomb testing as tracers for groundwater recharge studies, which is another example of the continuing effort of people of good will around the world to beat swords into ploughshares.

## 2. CALIBRATION AND USE

Since the 1950s, NMMs have improved in portability, programmability, weight and size. The advent of more-efficient detectors and the availability of Americium-241 produced in fission reactors resulted in the use of safer radioactive sources. The theory of operation of NS gauges and field calibration methods are described in several publications including Refs. [35] and [36]. The precision of measurements possible with NS has always been high and satisfactory for many soil water investigations with standard error  $<0.01 \text{ m}^3 \text{ m}^{-3}$  [6,35].

However, careful calibration and use remain essential to accurate soil water measurement with the NMM. This is particularly true because an important application of the NMM is to measure crop water use, where the primary aim is not to measure water content of soil, but the change in water content over a time period. Gardner [35] analysed variance in  $\theta_v$  due to the variance of neutron counts (ignoring errors in the slope and intercept of the calibration equation), and on that basis stated that the NMM measures change in water content less precisely than it does water content. He then stated that, for change in water content measurements, because “variations in parameters  $a$  and  $b$  (N.B. the regression coefficients) usually are not involved as in water content measurements ... the overall accuracy is better.” While it is true that the variances of the intercept and slope drop out of the calculation of the variance of a water content change measured at a single point in the field, this generally does not ensure the accuracy of change in water content values. In general, error estimation for changes in water content must include the error associated with the slope of the regression equation [37]. Only under two conditions is the accuracy of the change in water content assured. Errors in the calculated change in water content vanish only:

- for the trivial case of no change in the water content (even if the water content from the NMM is incorrect), and
- if the calibration error is in the intercept and not in the slope.

Neither condition is very likely. Many dry-land and irrigated crops will end the season with less stored soil water than at the beginning. Moreover, calibration errors are at least as likely to involve the slope as the intercept. In fact, in principle the intercept is a more conservative measure, reflecting only the mean of measured water contents.

Two common failures in NMM calibration are the failure to obtain a spread of water contents large enough to cover the expected range of water contents during field operations and large enough to accurately determine the slope of the calibration equation, and the combining of data from soil horizons with different neutron scattering and absorption properties into a single regression analysis. In the latter case, reported errors in slope have ranged up to +24% [37] and -59% [38]. Hignett and Evett [38] examined the error possible if data from the loam and clay horizons of a duplex soil in Australia were combined in a single calibration (Fig. 1, Table I). The combined regression will give

water contents that are close to actual values in the clay horizon, but will be far from correct in the loam horizon (errors greater than  $0.05 \text{ m}^3 \text{ m}^{-3}$ ). As the soil wets and dries, the reported change in profile water content will be in error due to the slope error in the combined calibration regression when applied to the loam. To be sure, if data as clearly different as those shown in Fig. 1 are available, then most practitioners would perform separate regression analyses on the data for the clay and loam horizons. However, the data shown are the result of repeated careful volumetric sampling at several different locations and water contents in each horizon. For less-careful and -complete sampling, it is easy to envision that scatter in the data or a reduced range of water contents encountered would preclude the observer from seeing that the data come from distinctly different populations. The reduced sensitivity of the method in clay illustrated in Fig. 1 is due to non-water hydrogen contained in the clay. The amount of non-water hydrogen reported as water content equivalent ( $We$ , g/g) can be considerable as shown in Ref. [36]:

$$We = 0.124 (\pm 0.012)C + 0.015 \quad (4)$$

where

$C$  is fraction of clay content of the soil in g/g.

Non-water H was measured as the water released from initially oven-dry soil heated to  $600^\circ\text{C}$  in an oxygen atmosphere for eighteen Australian clays.

TABLE I. REGRESSION EQUATIONS OF WATER CONTENT ( $\theta_v, \text{m}^3 \text{ m}^{-3}$ ) vs. COUNT RATIO ( $C_R$ ) FOR FIG. 1 (DATA FOR AN AUSTRALIAN DUPLEX SOIL [38])

Texture	Depth (m)	Regression equation	RMSE <sup>a</sup>	$r^2$ <sup>b</sup>	N <sup>c</sup>
Loam	0.3	$\theta_v = -0.037 + 1.150C_R$	0.005	0.99	6
Clay	0.6	$\theta_v = 0.060 + 0.513C_R$	0.010	0.96	6
Combined	0.3–0.6	$\theta_v = 0.084 + 0.472C_R$	0.027	0.85	12

<sup>a</sup>Root mean squared error. <sup>b</sup>Coefficient of determination. <sup>c</sup>Number of samples.

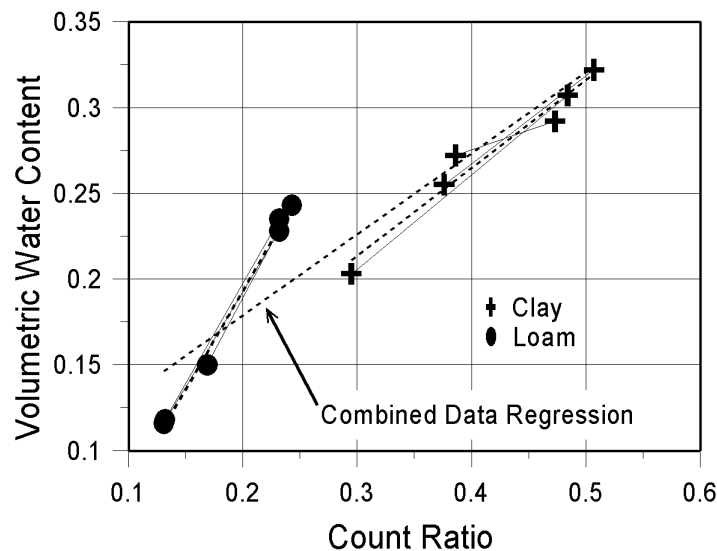


FIG. 1. Field calibration in a soil with variable texture using three pairs of access tubes in each texture. Regressions (broken lines) show clear differences in slope for loam and clay soils. The common regression shows a similar slope to the clay (offset by  $\sim 0.02$ ), but is biased for the loam. Profile water content change calculated using the common calibration will be considerably in error.

Because soils often are wetter at greater depth, a common mistake is to derive the desired range in water contents from samples taken at different depths. If this is done, differences in calibration coefficients due to water content differences are often confounded with differences due to soil materials because soil materials commonly change with depth. The degree of spread in the water contents has a direct effect on the calibration equation  $r^2$  value and thus the proportion of the variability in water content that is explained, through the calibration equation, by variations in count ratio. This is illustrated in Fig. 2. In Fig. 2a, the original data for a wet and dry site calibration are shown along with the calibration equation, which had  $r^2$  of 0.967 and SSE of  $0.014 \text{ m}^3 \text{ m}^{-3}$ . In Figs. 2b and 2c the wet end data points have been moved closer to the dry end points. The relative positions of the points have not been changed and they have all been moved an equal distance along a line whose slope is equal to the regression slope for the unaltered data. Thus, the degree of noise in the data due to noise in counts or in volumetric water contents has not been altered. This fact is reflected in the standard error of estimate, which remained the same at  $0.014 \text{ m}^3 \text{ m}^{-3}$  for regressions on the altered data sets. But, the intercept became increasingly more negative and the slope more positive as the range of water contents decreased. For Fig. 2b, the differences in slope and intercept were not large, but, for Fig. 2c, the slope increased by 0.039. This represents an error of about  $0.04 \text{ m}^3 \text{ m}^{-3}$  over a range of 1 in count ratio, which is equivalent to a water content range of about  $0.26 \text{ m}^3 \text{ m}^{-3}$  for the original data, or about a 16% error rate. The apparent invariant width of the 95% confidence intervals is misleading. Although the confidence intervals around the data points do not change, the confidence intervals outside the range of the data points (not plotted) increase dramatically. Thus, another advantage of a wide range of water contents is greater confidence in subsequent measurements over the range of water contents likely to be encountered in the field.

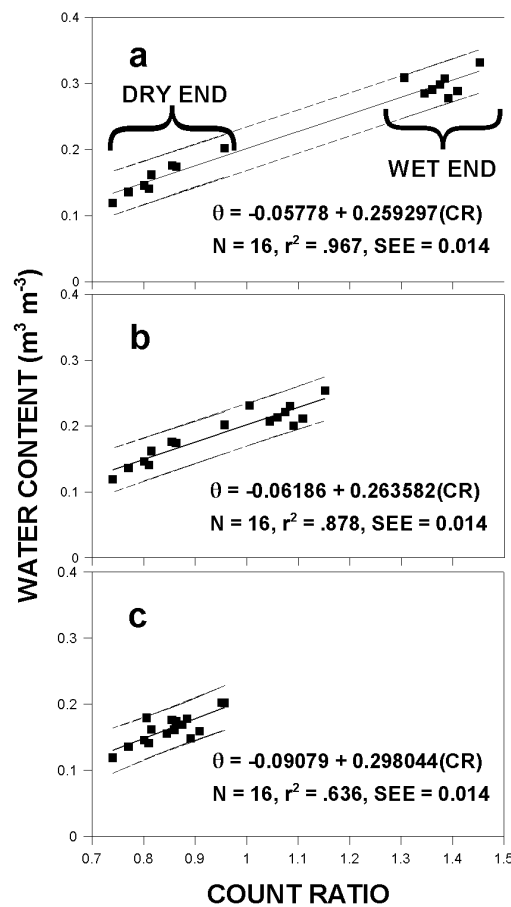


FIG. 2. An unaltered set of data from a wet site-dry site neutron probe calibration (a), and calibrations for the same data but with the wet end points moved closer (b) and still closer (c) to the dry end by sliding them along the regression slope. In each plot, the middle line is the regression line and the upper and lower lines are the 95% confidence intervals.

The following discussion will concentrate on recommendations for use and calibration. Some of it is drawn from a chapter on the neutron thermalization method [38].

## 2.1 Statistics of neutron emission

Neutron emission is a random process that occurs according to a Poisson probability distribution. An important property of the Poisson distribution is that, for a series of counts over equal time periods, the standard deviation is equal to the square root of the mean value. The sample mean,  $m$ , is computed as

$$m = \frac{1}{N} \sum_{i=1}^N x_i \quad (5)$$

where

$x_i$  is the value of a single count,  
 $N$  is the number of counts.

The sample standard deviation,  $s$ , is computed as

$$s = \left[ \frac{1}{N-1} \sum_{i=1}^N (x_i - m)^2 \right]^{1/2} \quad (6)$$

For a properly operating gauge with the probe in a constant environment, the ratio of  $s/(m)^{1/2}$ , called the Chi ratio, should be close to unity. This ratio is related to the  $\chi$  statistic by

$$\frac{s}{m^{1/2}} = \left( \frac{\chi^2}{N-1} \right)^{1/2} \quad (7)$$

Values of  $\chi^2$  for a given probability level are given in statistical tables for different values of  $(N-1)$ . We may write the right-hand side of Eq. (7) for the upper and lower limits of  $\chi^2$  and thus obtain upper and lower values of the Chi ratio for the chosen probability level and number of samples. For example, for a 95% probability level and thirty-two samples, we find the values of  $\chi^2$  as 17.5 for  $P = 0.975$  and 48.1 for  $P = 0.025$ ; and from Eq. (7) the Chi ratio should be between 0.75 and 1.25 about ninety-five times in every hundred. Note that some gauges divide the count by a fixed number in order to reduce the displayed count to a reasonably small value. If the above calculations are applied to such reduced counts, the Chi ratios computed will be incorrect. To compute Chi ratios, the user should first multiply the recorded counts by the factor that the gauge used to reduce them.

These facts allow the user to check gauge operation by computing the Chi ratio. In fact, all modern gauges include an internal function for doing so, either through a special “STAT” function, or as a normal part of taking a standard count (see below). The range of the Chi ratio for a particular gauge will depend on the number of samples that that particular gauge uses in computing the standard count and Chi ratio values. An occasional Chi ratio outside of the 95% range is no large cause for concern, but should be checked by making another test. However, a series of Chi ratio values that average above or below unity signals a problem in the gauge electronics or internal geometry, and indicates inaccurate readings. For this reason, a quality assurance program for NMM measurements should include daily measurement and recording of the standard count and Chi ratio.

## 2.2 The standard count

While modern instruments are quite reliable, it is good operating practice to measure the count rate in a standard medium at regular intervals (i.e. daily) to check the machine for faults. Even if the Chi ratio remains near unity, a sudden change in the standard count signals a problem with the gauge that should be corrected before more field readings are taken. Problems that can occur include failure of electronic components, cable and connector failures, and detachment of the detector tube inside the probe (change in geometry of measurement). For profile meters, an ideal standard is a 200-L container (or, at least 0.55 m diameter and 0.8 m height) filled with distilled water and equipped with a central access tube of the same material and size as is used in the field [39]. The centre of measurement of the

probe should be located in the centre of the volume of water; and the access tube should extend to the bottom of the container to avoid the influence of nearness of the end of the tube.

Many users prefer the convenience of using the small plastic shield in the meter body as a standard. In this case the count can be affected by nearby bodies. However, it is acceptable provided the shield is mounted at least 0.8 m above the soil surface during the count (Fig. 4 in [40], Fig. 1 in [41]), and at least 3 m from surrounding objects. The operator should stand at least 3 m away to avoid influencing the count. In a 10-year study, probe shield counts were found to be reliable and highly precise [42,43]. The depth control stand described above is useful for this purpose. Neutron probe calibrations that used the depth control stand for standard counts resulted in values of standard error  $<0.01 \text{ m}^3 \text{ m}^{-3}$  [44]. Even though recommended by some manufacturers, the practice of placing the meter on its case for the count is to be avoided because the case is not of sufficient height to avoid the influence of materials below the case. Although placement of the meter on the tailgate of a vehicle may raise it to the necessary height above the soil, the nearness of neutron absorbers and moderators in the body and fuel tank of the vehicle makes it poor practice.

### 2.3. The count ratio

Calibration equations for surface gauges and profile meters are typically linear functions of a count ratio ( $C_R$ ). The value of  $C_R$  is the ratio of the count,  $x$ , made in the measured material to a standard count,  $x_s$ .

$$C_R = x/x_s \quad (8)$$

It is good practice to express the calibration in terms of the count ratio. Using a calibration based on count ratio rather than in terms of direct counts, allows the same calibration equation to remain valid even as the source strength decays. The half-life of  $^{137}\text{Cs}$  is 30.6 years and that of  $^{241}\text{Am}$  is 458 years. To maintain 0.1% accuracy for density measurements ( $^{137}\text{Cs}$ ), the standard count must be re-determined every 2 weeks. To do the same for moisture measurements ( $^{241}\text{Am}$ ), the standard count must be re-determined every 8 months. By use of the standard count and count ratio, an accurate calibration may be used for many years. To reduce random error, it is recommended that the standard count used in Eq. (8) be a running average of the previous ten standard counts. This is particularly important for calibrations or for repeated measures used to determine crop water use over small time intervals.

### 2.4. Access tube materials

Aluminium is recommended for access tubes because it has minimal effect on neutrons. However, it is relatively expensive, not particularly strong and can quickly corrode in saline or alkaline conditions. Mild steel tubing is inexpensive, strong, and will last for at least 3 years in all but very acid soils. It has the disadvantage that it absorbs neutrons and decreases sensitivity of the instrument by about 2% but with no apparent effect on accuracy, hence may have the apparent effect of slightly increasing calibration error. Stainless steel can be used, but is expensive and also decreases sensitivity. Plastics including polyethylene have been used successfully and have the advantages that they are slightly flexible (useful in stony soils) and inexpensive. However, the density and wall thickness of plastic tubes vary, particularly between batches, which introduces a small additional random error. Calibration must be done using material of the same batch as the main installation. The root mean squared error of regression increased by  $0.003 \text{ m}^3 \text{ m}^{-3}$  when PVC rather than Al tubing was used by Allen et al. [45]. Neutron count values were ~50% larger for Al vs. PVC tubing in two studies, due to neutron capture by the chlorine atoms in the PVC [41,45]. Neutron counts obtained using steel tubing are lower than those obtained when using Al, but are closer to counts with Al than to counts with PVC [46]. Thus, the NMM is slightly more sensitive to water content changes when using Al or steel tubing than when using PVC tubing. Coefficients of determination ( $r^2$ ) values decreased consistently but nearly negligibly by 0.006 to 0.013 when steel rather than Al tubing was used for field calibrations of four types of NMM [47]. Accurate and precise calibration and water-content measurement are much more dependent on other factors explained below than on the material used for access tubing.

## 2.5. Access tube dimensions

The access tube should be as small as possible to maximize sensitivity, but have sufficient tolerance to allow the probe to move freely, even if there are small distortions in the tube. Tubes of 44 to 56 mm outside diameter and walls of 1.6 mm thickness are used commonly. Both larger diameter and greater wall thickness may result in decreased meter counts [41]. Larger diameter tubes are used with larger diameter probes. The larger diameter tends to increase the radius of the volume of influence and decrease the vertical resolution of the instrument [8]. Access tubes should be about 0.3 m longer than the greatest depth to be measured. This allows 0.15 m extra at the bottom to prevent the deepest reading from being influenced by the soil at the end of the tube [48], and 0.1 to 0.15 m of tube to extend above the surface to make the tube visible in the field and to prevent accession of surface water. The desirable maximum depth will depend on the particular study. But in studies to determine crop water use, it should be well below the maximum depth of rooting and below any expected zero flux plane for soil moisture [26]. Protrusion from the soil should be minimized to prevent rainfall collection and heat conduction from the soil surface.

## 2.6. Removable extensions

For long-term installations where tillage is needed, the tube is constructed so that the top section (usually 0.3 m) is held in place by a slightly oversized sleeve. The top section of tube is removed before tillage and the tube is plugged. Methods of locating tubes after tillage include measurements from fixed reference points, the use of a metal detector, and the use of coloured wire attached to the tube before burial. There may be problems with calibration where the extension attaches (extra wall thickness), with soil disturbance adjacent to the tube, and with water leaks if the soil becomes saturated. However, tube extensions are routinely used successfully.

## 2.7. Control of probe depth placement and shield height

It is common practice to place the NMM on top of the access tube before releasing the probe from the shield and lowering it for readings in the soil. This practice is not recommended for two reasons. First, when the NMM is placed on top of the access tube, the shield in the meter body may influence near-surface counts to a degree that depends strongly on the height of the meter above the soil [48]. Second, in field use, the actual height of access tubes above the soil is likely to change with tillage, rainfall induced compaction, erosion or deposition, or other factors, resulting in an equivalent change in the depth of probe placement. For readings at depths of less than 0.2 to 0.3 m, the depth of the probe will influence the reading and the calibration due to loss of neutrons to the atmosphere [9,49,50]. Hauser [50] found that the calibration equation intercept became more positive as depth decreased but that the slope remained constant. However, Evett [44] found that the slope became smaller and the intercept larger as depth decreased.

These problems are addressed by using a depth-control stand. This device comprises a length of access tube fixed to a 0.2-m length of slightly larger tubing that is, in turn, supported by a foot resting directly on the soil (Fig. 3). The larger diameter of the lower length of tubing allows it to be slipped over the top of an access tube so that the foot rests on the soil surface. This maintains the reading depth at an exact distance relative to the soil surface. Cable stops are arranged to achieve the desired depth placement of the probe. Instructions for fabricating and using a depth-control stand are available<sup>2</sup>. The stand described is tall enough to be suitable for taking standard counts with the NMM mounted on the stand and the probe locked in the gauge shield (see below).

---

<sup>2</sup>Evett, S.R., Construction of a Depth Control Stand for Use with the Neutron Probe, USDA-ARS-CPRL, Bushland, TX, <http://www.cprl.ars.usda.gov/programs/> (posted July 2000).

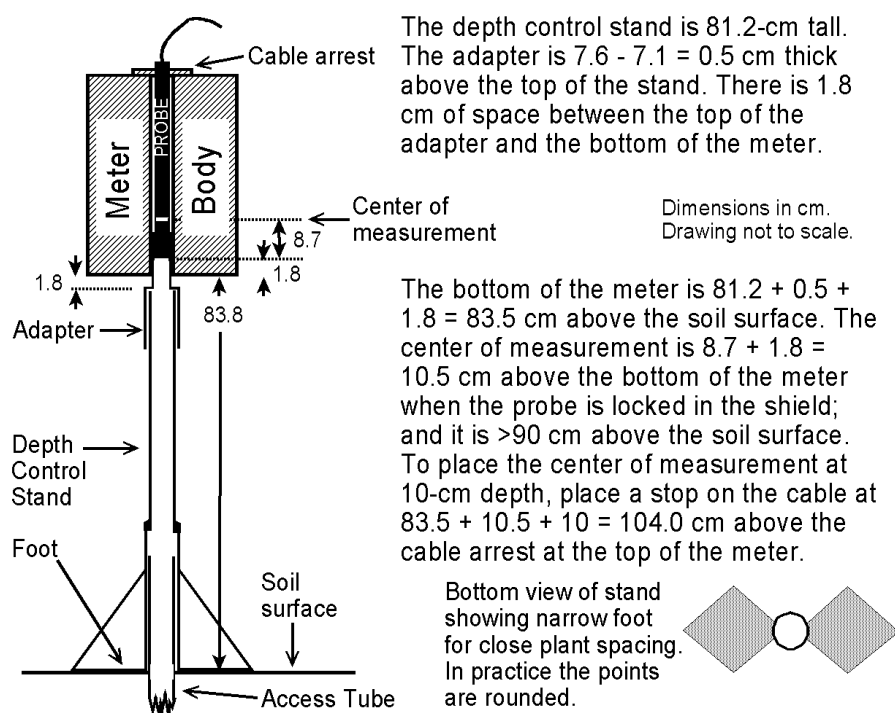


FIG. 3. Example dimensions relevant to placing cable stops on cables to achieve accurate depth placement of the probe centre of measurement when using a depth-control stand.

## 2.8. Access tube installation

Neutron moisture meters are relatively immune to installation problems because the soil volume affected by access tube placement is small compared to the volume of measurement. The tube needs to be tight fitting with no cavities around the tube, both because the calibration depends on the cavity size [41], and to prevent free surface water from leaking down the tube. A rubber stopper or inverted tin can should be used to prevent rain and animals from entering the tube. It is also a good idea to clearly mark the tube with a number to aid identification. Several methods of installation are commonly used.

### 2.8.1. Push and sample

The tube is inserted a short distance into the soil and pushed down in shallow lifts while augering from inside the tube. Augering is limited to a few centimeters in advance of the tube. For this method, the tube should be beveled on the inside so that all soil is pushed toward the center as the tube is pushed down. This method provides the best results, minimizing compaction of soil near the tube and voids between the tube wall and soil, but is very difficult and time consuming if done manually.

### 2.8.2. Sample and ream

A hydraulically driven push tube or hand auger is used to dig a slightly undersized hole. Then the access tube is pushed into the hole and an auger used to clean out the excess soil by extracting it from inside the tube. Hand augering is preferable to machine augering or push tube sampling to minimize soil disturbance around the tube. It is difficult to keep the tube from wandering from the axis of the hole during insertion, which leads to voids between the outside tube wall and the hole. To keep the access tube centred in the undersized hole, put a bevel on the outer edge of the access tube. In this case, the hole diameter should be only very slightly smaller than the tubing outside diameter. If a push tube is used to install access tubes it may cause compaction around the tube, particularly if the bit on the push tube is bevelled on the outside, and if installation is into a moist, compressible soil.



### 2.8.3. Slurry

A slightly oversized hole (2 mm) is dug using an auger or push tube. The access tube is sealed at the bottom, and a slurry of local soil with 10% cement as a binder is poured down the hole and the access tube quickly inserted so that the slurry rises to fill the gap around it. The slurry should be as dense as possible to minimize shrinkage on drying. If local soil is unsuitable, a mixture of 10% cement, 40% kaolin clay, and 50% water (by weight) can be used. The clay in this mixture will affect the calibration, necessitating a field calibration procedure, particularly if the local soil is low in clay content. This method is recommended except where extreme accuracy is required. Note that the shrink/swell clay bentonite should not be used. It will shrink on drying, leading to the possibility of free water travelling down shrinkage cracks immediately adjacent to the access tube.

Note that with most tube installation methods, the soil sample extracted from the hole may be used for calibration checks. If there is a risk of water entering the tube from below, the tube should have a rubber plug, a hydraulic cement seal (poured down a tube inserted inside the access tube), or other watertight material. Traffic by vehicles during installation should be controlled to ensure that access tubes are not installed in wheel tracks. Compaction by foot traffic in the immediate vicinity of an access tube should be avoided. This may require the use of a protective platform, such as a pallet during installation.

### 2.9. Vertical reading interval, maximum reading depth, and number of tubes

Design of a NMM installation is a compromise between the number of tubes needed for a sufficiently precise measure of soil water and the cost of installing and reading the network of access tubes. Modern instruments can take a reading of adequate precision in 30 seconds. Readings are often taken at 0.2-m intervals. Therefore ten readings, or 5 min are needed to scan a single tube to 2 m depth. If tubes are located close together then a reading rate of ten tubes per hour is a reasonable goal for a single instrument and operator. The number of tubes that can be read in a day will decrease as travel time to the site and between tubes increases.

From Eq. (1), the depth range of each measurement can be assumed to be at least 0.15 m above and below the reading point. In reported field studies, the depth interval between readings has been as small as 0.15 or even 0.10 m [27,51]. Depth intervals should not be larger than 0.15 m in soils with large and rapid changes in water content with depth [8, 52–54]. Intervals as small as 2.5 and 7.5 cm did not improve the accuracy of soil moisture measured in a profile with a large and rapid change in water content [54]. Although water contents near such interfaces are incorrect, the errors tend to compensate on either side of the interface if small intervals ( $\leq 15$  cm) are used; and calculations of change in water content over time remain accurate [54]. Intervals of larger than 15 cm can cause larger errors [54]. A depth interval of 0.10 m was reported to improve precision of profile water content [27], but the improvement was likely due to the increased counting time resulting from the additional readings rather than any new information about the profile [55]. Depth intervals of greater than 0.3 m are sometimes used where water content changes slowly with depth. However, this is done at the risk of losing some information as the effective range of the NMM may be only 0.15 m from the probe in wet soil. The desirable maximum depth of reading will depend on the particular aims of a study. But in many studies it should be well below the maximum depth of rooting and below any expected zero flux plane for soil moisture [26].

The number of samples required to measure water content to a given precision depends on the sample size and on the spatial variability of water content both horizontally and vertically. Variability tends to increase as sample size decreases, particularly for samples smaller than the representative elemental volume. Water content varies as a function of soil properties, topography, climate, tillage, and the type and growth stage of any plants. Decisions about the number of access tubes needed to achieve an acceptable precision require additional information about the soil and plant environment to be studied. If previous experimental work has been done, then one may use information about site variability from that work. References dealing with variance and spatial variability of NMM measurements include

Haverkamp et al. [56], Sinclair and Williams [57], Vandervaere et al. [51,58], and Vauclin et al. [59]. The NMM measures, at minimum, a volume of  $\sim 0.014 \text{ m}^3$ . Comparing this with the much smaller sampling volumes of most time domain reflectometry (TDR) and capacitance probes gives an idea of how many measurements would be needed with these technologies to give a field or plot mean profile water content with precision comparable to that from neutron thermalization. Approximately twenty-four soil samples (50 mm diameter and 0.3 m long) would be needed to sample the same soil volume as one NMM measurement. This fact explains why calibration methods that fail to provide multiple soil samples for every neutron probe reading tend to be imprecise. Variance in soil properties can also affect the precision of the calibration of the NMM (see below).

## 2.10. Calibration

Contrary to Gardner [35], manufacturers' calibration equations are seldom useful for routine soil moisture determination [60–62]. Calibration of NMMs involves correlating measured count ratio values with independently determined volumetric water contents,  $\theta_v (\text{m}^3 \text{ m}^{-3})$ . For modern gauges and the normal range of values of soil water content, the calibration is linear and of the form

$$\theta_v = b_0 + b_1 C_R \quad (9)$$

where

$b_0$  and  $b_1$  are the calibration coefficients as determined by linear regression, and  $C_R$  is the count ratio defined above.

Because of the wide variety of agricultural soils, it is necessary to calibrate for specific soils even though NMMs come with factory calibrations. If possible, it is best to calibrate in the field so soil horizon-specific calibration coefficients may be determined. Otherwise, fairly large errors may result because calibration coefficients vary widely for horizons rich in clay, organic matter,  $\text{CaCO}_3$ ,  $\text{CaSO}_4$ , or close to the surface. Several calibration methods are commonly used. Each has qualities that recommend it for a particular use.

### 2.10.1. Method 1: laboratory method for a uniform soil

If a soil is uniform to considerable depth, a laboratory calibration may be appropriate. Excavate approximately  $2 \text{ m}^3$  of soil from the field and transport it to the laboratory. Air dry and crush it to pass a 5-mm sieve. Mix it thoroughly and pack into a steel container of at least 1.22-m both in diameter and height (to obtain an equivalent infinite volume [9]), open at top and bottom and divided so that it can be split vertically into two halves to simplify removal of the soil. An access tube of the same composition and size as used in the field is mounted in the centre of the container.

Add air-dry soil to the container in quantities of 40 kg, spread evenly, and lightly pack. Obtain NMM counts at four depths (the container centre point, at 0.1 m above and below it, and at 0.1 m depth) for five times the normal counting period. At the same depths, take at least five volumetric samples for gravimetric water content and density. Split the container into its two halves and remove the remaining soil, crushing any cohering lumps. If the soil has  $>2\%$  organic matter or clay then it should be repacked using a greater packing pressure (a smaller rammer) to achieve a higher density and the count rate again taken to measure the effect of density and non-water H. The soil is then removed, crushed and distilled water is sprayed onto it to achieve about a 5% increase in water content, and the soil thoroughly mixed and left to stand overnight under a cover. After mixing again, the container is repacked, and the NMM readings repeated for the higher water content (at high and low density if necessary), followed again by volumetric soil sampling. This process is repeated until the soil is too wet to work. For each reading depth, the volumetric water content of the packing is estimated from the average density and the gravimetric water content of the soil and plotted against the count ratio of the NMM. For each packing, the data for the centre depth and 10-cm above and below it may be combined. Data for the 10-cm depth are used for a separate near-surface calibration. A linear regression is then fitted to the data points using the variable (count ratio or water content) with the least error as the dependent variable. This is because regression theory requires that the independent

variable be known precisely; and both NMM count and water content have error [63]. If water content is known more precisely, and is used as the independent variable, then the equation can be inverted before use. If the high- and low-density points are fitted separately, then the effect of density and volumetric non-water  $H$  (which changes with density) can be measured also.

Laboratory calibration can result in regression equations with low values of standard error of regression. But, it is difficult to pack soil to field bulk density at all water contents, the method is time consuming, and it may be difficult or impossible to account for soil-horizon-specific variations in NMM response that require separate calibrations. Also, it is never clear that laboratory calibrations reflect field conditions.

#### 2.10.2. Method 2: field calibration for uniform soil or soil horizons

This is the most common calibration method. A calibration should include as wide a range of soil water contents as possible. It is best to establish both wet and dry sites in a field for calibration, either at the same time or sequentially (Fig. 4). A dry site may be established by growing a crop that normally dries the profile, and/or by waiting for a time of year when the soil is normally dry. A wet site may be established by berming an area and ponding water on it until the wetting front has descended below the lowest horizon to be calibrated. If the soil is a heavy clay it may take many days to wet up. Typically, three access tubes will be installed at each of the dry and wet sites with at least a 1-m spacing between tubes. Prior to using the NMM, check the depth stops on the cable to ensure accurate depth positioning (Fig. 3). Take at least three standard counts, and check counts and Chi ratios for stability. Take 60-second or longer counts at all the depths required for calibration. For the wet site, wait an appropriate time after ponded water is removed in order to allow rapid internal drainage to take place before taking counts. This ensures that soil water content does not change appreciably between the time counts are taken and the time that soil samples are obtained. For the same reason, it is important to obtain the gauge readings and soil samples for the wet site on the same day.

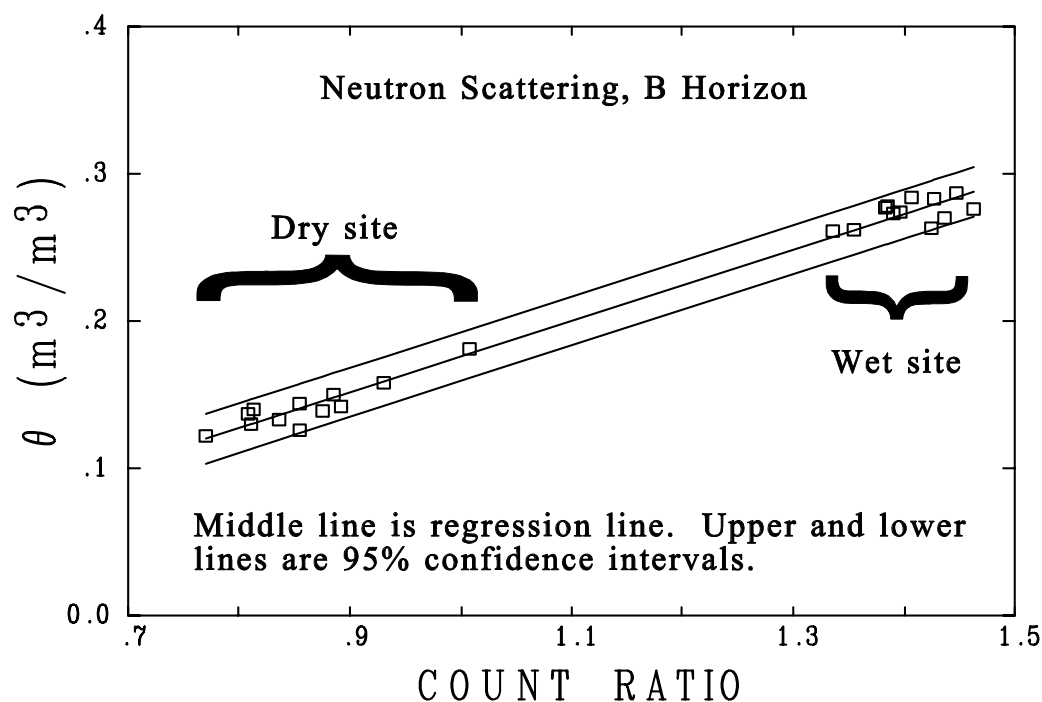


FIG. 4. Typical differences in water content between wet and dry profiles and calibration line from a wet-site/dry-site calibration.

Take at least four volumetric soil samples at each depth around each tube. Sample around the wet site tubes immediately after counts are taken. A volumetric soil sampler such as the Madera probe [44] (Fig. 5) or thin-walled metal cylinders should be used (Fig. 6) [64]. Use of thin-walled samplers to obtain undisturbed samples usually requires a clearance ratio  $(D_i - D_e)/D_e$  of 0.01 or larger (Fig. 5) [64]. Open-ended samplers such as these are recommended because they allow the user to observe the sample condition after the sampler is inserted in the soil, and thus allow checking for soil compression or shattering during insertion. Closed-end samplers, with or without removable metal sample cylinders, are to be avoided because of potential compression. For tube samplers of 50-mm diameter, a wall thickness of no larger than 1.2 mm is desirable [64]. This gives a ratio of cutting-edge surface to sample surface of 0.05 (surface areas normal to the axis of insertion). To lessen compaction, the sample surface area should be at least 90% of the area of the hole created by the sampler. A minimum of four samples should be taken from within 0.1 m of the tube at the reading position (that is, sample from the volume of soil measured by the probe). Take care when removing the soil near the tube so as not to disturb the remaining soil before the remaining samples are taken (avoid compaction or soil loosening). If using the Madera probe or a similar tube probe, samples may be taken horizontally from the side of a pit dug on one side of the access tube. Alternatively, the soil may be removed to a depth about 0.1 m above the reading depth and the samples taken vertically (Fig. 6). To calibrate at depths greater than 0.5 m it is best to dig a trench not closer than 0.5 m from the tube, then work from the trench to excavate to the sample depth. While taking soil samples, note depth of any soil horizons that might lead to different calibrations. Before regression analysis, calculate the mean and standard deviation of soil water content and bulk density for each depth at each access tube. Examine these data and recompute the means after removing obvious outliers. For example, in Table II each of the N values is the mean of four samples taken at a particular depth at a particular access tube (six samples for the 10-cm depth). This method is designed to provide a mean soil water content for the volume of soil that is read by the NMM. It can routinely produce root mean squared error values (RMSE) for linear regression of  $<0.01 \text{ m}^3 \text{ m}^{-3}$  (Table II) [44].

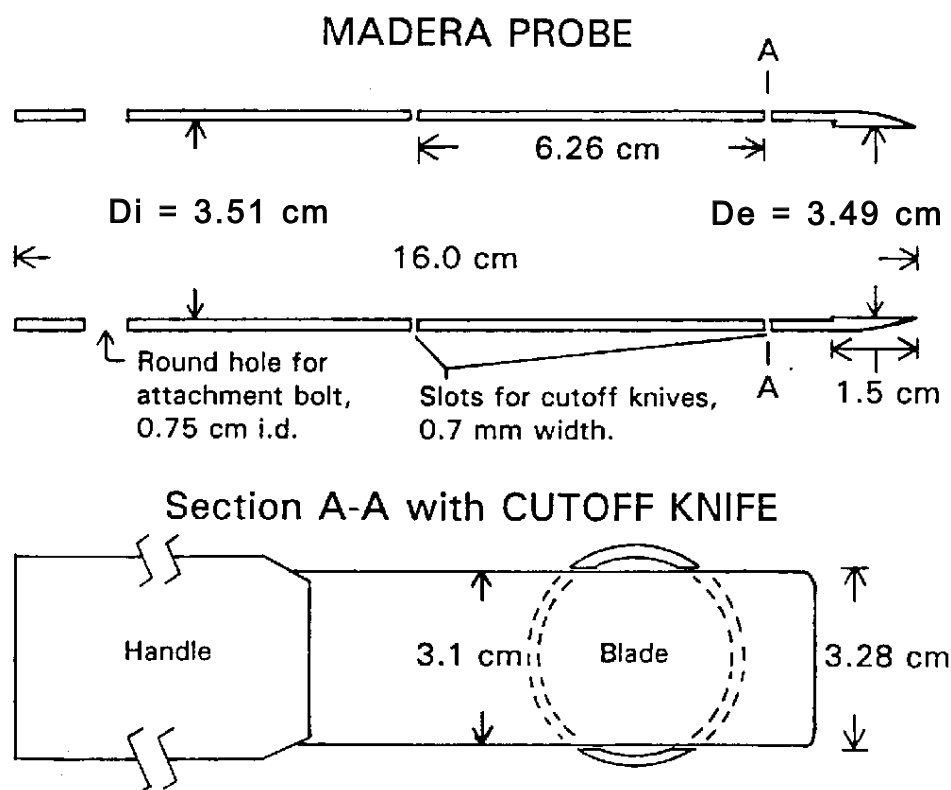


FIG. 5. The Madera probe may be obtained from Precision Machine Company, Inc., 2933 North 36th Street, Lincoln, NE 68504-2498 USA, tel 402-467-5528, fax 402-467-5530.

A variant of this method was used by Allen et al. [45] and Dickey et al. [65] in which samples were taken with a 1.22-m-long tube probe pushed into the ground with a hydraulic ram. Wet and dry sites were used. The sample was extruded from the tube onto a tray and sectioned into lengths corresponding to the depth intervals measured with the NMM. The process was repeated to create a hole at least 1.5-m deep. The volume of each sample was calculated as the area of the cutting edge of the tube probe multiplied by the length of that section. Access tubes were installed in the holes created by sampling and NMM measurements taken at the centres of the depth ranges sampled. The method achieved the best results of the three methods they compared in three soils (which did not include method 2 described here), but also gave consistently higher  $C_R$  values, a result of compression of soil around the tube probe that was particularly evident in a clay loam soil. The RMSE of regression ranged from 0.006 to 0.013  $\text{m}^3 \text{m}^{-3}$ . This variant is not recommended in soils that compress easily as soil in the sampler may be compressed or dilated during sampling. The USDA-SCS (now NRCS) method is similar except that the Madera probe fitted to an extension tube is used to take a 60-cm<sup>3</sup> sample at each reading depth with hand augering to deepen the hole between samples [62,66]. In the study of Allen et al. [45] the RMSE of regression ranged from 0.011 to 0.014  $\text{m}^3 \text{m}^{-3}$  for the SCS method; but  $C_R$  values were not raised by soil compression. Both of these methods are designed for calibration during access tube installation so that the access tube may be used for subsequent moisture readings [45]. But, both have dual disadvantages:

- the soil sampled is removed from the hole before NMM counts are measured, and
- there is only one sample per depth associated with each NMM count.

The accuracy of this variant can be improved considerably by taking cores at three or more places around the access tube and close enough to be within the measurement volume [26].

TABLE II. CALIBRATION OF WATER CONTENT  
( $\theta_v$ ,  $\text{m}^3 \text{m}^{-3}$ ) vs. COUNT RATIO ( $C_R$ ) FOR THE  
AMARILLO FINE SANDY LOAM WITH METHOD 2,  
USING A DEPTH-CONTROL STAND (FROM [67])

Depth (cm)	Regression equation	RMSE <sup>a</sup>	$r^2$ <sup>b</sup>	N <sup>c</sup>
10	$\theta_v = 0.014 + 0.2172C_R$	0.004	0.997	6
30–190	$\theta_v = -0.063 + 0.2371C_R$	0.007	0.988	44
30–90	$\theta_v = -0.066 + 0.2421C_R$	0.008	0.988	24
110–190	$\theta_v = -0.057 + 0.2299C_R$	0.006	0.992	20

<sup>a</sup>Root mean squared error. <sup>b</sup>Coefficient of determination. <sup>c</sup>Number of samples.

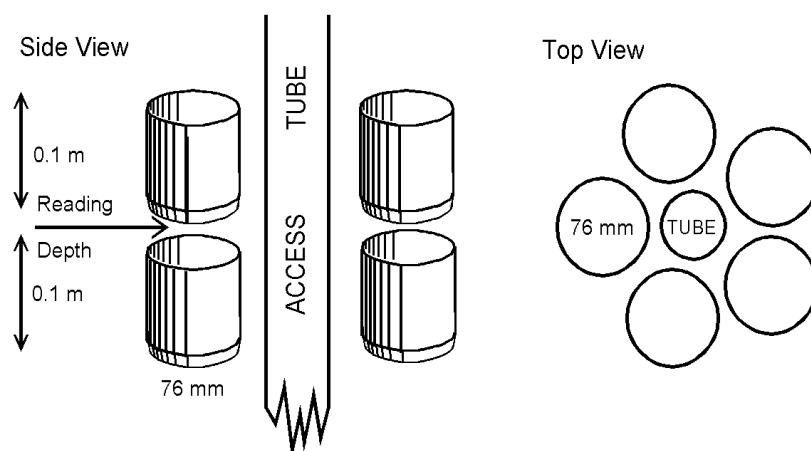


FIG. 6. Placement of volumetric sampling cylinders for optimum sampling of the NMM measurement zone.

### 2.10.3. Calibration for near-surface readings

The loss of a substantial fraction of neutrons from the surface during near-surface readings (<0.3-m depth for fine-textured soils, <0.38-m depth for sands [54]) necessitates a separate calibration for any depths in this zone. Special techniques for surface-layer calibration are described by Sartz and Curtis [60], Grant [49], Parkes and Siam [37], and Hauser [50]. However, the ordinary field calibration procedure (method 2) is quite adequate provided a depth-control stand is used (see calibration for 10-cm depth in Table II).

### 2.11. Transferring calibration coefficients between meters/checking meter calibration

Calibration of NMMs is time consuming whether done in the laboratory or the field. It is desirable to be able to transfer the calibration between a calibrated meter and a new or repaired one, and to be able to check a calibration. There are both field and laboratory methods for transfer.

For the field method, one identifies or creates soil profiles having large differences in water content, similar to the wet and dry sites created for a field calibration (see above). The calibrated meter is used to determine water contents at various depths in one or more access tubes both in the dry and the wet profiles. If separate calibrations exist for different soil horizons, it is prudent to have at least three access tubes in each site. Counts in these tubes with the meter to be calibrated or checked are used to compute count ratios for that meter corresponding to each depth, and the transfer calibration is established by linear regression vs. water contents from the calibrated meter [47]. Care must be used to establish the centre of measurement at equivalent depths for both meters. Unlike transfer using laboratory standards, the field method works well for transfer of calibration between meters of different manufacture or internal design (S.R. Evett, unpublished data).

In the laboratory, calibration transfer can be done by preparing drums of media with sufficient volume such that counts would not increase if further volume were added (quasi-infinite volume) and with media characteristics that produce widely different count numbers. Once a calibration is transferred to such a set of drums, the calibration may be transferred to NMMs of the same design, with some precautions [68]. Meters from different manufacturers have different source-detector configurations and respond to these media differently so that, in general, calibrations cannot be transferred between them. Even for a single manufacturer and model number, if the NMMs were manufactured many years apart, the internal characteristics may be sufficiently different that calibrations cannot be transferred in this manner (S.R. Evett, unpublished data). In any case, once a calibration is transferred, the NMMs involved should be used to obtain soil water content values in wet and dry sites in the field and those values checked for correspondence across meters. Of course, the transferred calibration will be valid only for the soil in which the initial calibration was done.

Media used have included water, aluminium sulphate ( $\text{Al}_2(\text{SO}_4)_3 \times 18\text{H}_2\text{O}$ ), urea ( $\text{NH}_2\text{CONH}_2$ ) [39,69], ammonium alum ( $\text{Al}_2(\text{SO}_4)_3(\text{NH}_4)_2\text{SO}_4 \times 24\text{H}_2\text{O}$ ) [70], and high-density polyethylene plastic cylinders of various radii [68]. Nakayama and Reginato [47] found that water contents were more accurate by up to  $0.03 \text{ m}^3 \text{ m}^{-3}$  when using a field transfer method than when using plastic cylinders. Depending on its size and constitution, each medium will represent a particular equivalent water content. At least two such media are required, one with a small equivalent water content and one with a large equivalent water content. For long-term stability, the media should be prepared so that their properties do not change with time. For example, in the 10-year study of Stone et al. [43], urea was hygroscopic and took up water even though sealed in polyethylene sheeting and doubly sealed in a steel drum, while aluminium sulphate protected in the same way was quite stable. McGuinness et al. [70] were able to create media with equivalent water contents ranging from 0.033 to  $0.434 \text{ m}^3 \text{ m}^{-3}$  by mixing ammonium alum with silica sand, and found the resulting mixtures to be very stable “even when exposed to air.” If a medium does not represent a quasi-infinite volume (e.g., the plastic cylinders of Reginato and Nakayama [68]), then care must be taken to remove the meter shield and any other objects from the vicinity of the medium during counts.

To transfer a calibration to the media, a calibrated meter(s) is centred in each medium and a count is made for a sufficiently long time to minimize count variance. Standard counts are also obtained (see

above); and the equivalent water content for each medium is calculated from the calibration equation. Subsequently, the same meter, or a very similar meter, may be used to obtain counts in the media and standard counts, and a calibration may then be calculated using the equivalent water contents as the independent variable.

### 3. SOME CALIBRATION STUDIES

Stone et al. [71] conducted the ASCE (American Society of Civil Engineers) Neutron Probe Calibration Study on three agricultural soils, Millville silt loam, Nibley silty clay loam, and Kidman sandy loam. Sub-studies were done on methods of bulk density measurement, effects of the geometry of source and detector tube (source at bottom of detector, or source centred around detector), and effects of access tube material (aluminium, steel or polyvinyl chloride plastic). No attempt was made to produce calibrations for different soil horizons, probably because sample numbers were inadequate (they ranged from six to eighteen for the entire profile). Three access tubes were installed in a wet site and three in a dry site for each soil, with 10 cm of tube protruding above ground level. Sampling depths were at 15 cm below ground surface and in 15-cm increments below that to 150 m. Shield counts, used to calculate count ratios, were taken with the gauges resting on the top of an access tube at 1.5 m above the soil surface. Calibration equations were calculated by linear regression analysis of measured volumetric water content vs. count ratios.

A probe with the source centred around the detector tube (model 3223, Troxler Electronics Inc., Research Triangle Park, NC) showed greater sensitivity to water content than the probe with the source at the bottom of the detector (model 503DR, Campbell Pacific Nuclear (CPN) International, Martinez, CA) [48]. The two probes were equally sensitive to proximity to the surface. The centred detector-source probe showed slightly better resolution of vertical changes in moisture content and of a cavity placed in the soil adjacent to the access tube. Both probes were sensitive to placement above the bottom of the augered access hole. Changes were 1.64 standard deviation (SD) for the Troxler and 1.19 SD for the CPN, from readings with the probes about 10 mm above the bottom of the hole, when the hole was augered another 15 cm deeper and readings were taken at the same depth. This suggests that calibration efforts should ensure that the augered hole extends well beyond the lowest depth of reading. Despite the greater sensitivity of the Troxler probe, there was no significant difference in the precision of calibration curves developed for the two brands of gauges [72]. Standard errors of estimate ranged from 0.0068 to 0.0193 m<sup>3</sup> m<sup>-3</sup> for CPN gauges and from 0.0056 to 0.0197 m<sup>3</sup> m<sup>-3</sup> for Troxler gauges [45].

Access tube materials affected the calibration equation slope a great deal, but affected the intercept only slightly. Both brands of gauge were more sensitive to water content when used with aluminium tubing and least sensitive when used with PVC tubing. Sensitivity with steel tubing was in between that for Al and PVC tubing [72]. Calibration equation standard errors of estimate ranged from 0.0056 to 0.0147 m<sup>3</sup> m<sup>-3</sup> for Al access tubes and from 0.0111 to 0.0193 m<sup>3</sup> m<sup>-3</sup> for PVC access tubes, indicating a slight reduction in precision of calibration when using PVC tubes.

Three soil sampling methods for neutron probe calibration that do not destroy the site were compared by Allen et al. [45] and Dickey et al. [65]. Two were down-hole methods for which samplers were pushed into the soil at the bottom of an augered hole to take fixed volumetric samples. Of these, the SCS Madera sampler, with a 60-cm<sup>3</sup> sample volume, resulted in better calibrations (lower standard error of estimate) than the Utah State University sampler that had a smaller volume of 15 cm<sup>3</sup>. The third method, involving a Giddings coring tube, produced the smallest calibration error estimates. With this method the coring tube was inserted by a hydraulic coring machine (Giddings Machine Co., Fort Collins, CO) and the soil core was pushed out of the tube onto a tray where it was cut into sections of known length, which were placed in soil cans. Volume of each sample was calculated from the inside diameter of the coring tube cutting edge and the sample length. Use of the Giddings coring tube did result in compaction of the soil around the hole in which the access tube was subsequently installed, and this caused the calibration slope to change. Thus, although the calibration error estimate was smaller with this method of sampling, the calibration probably did not provide an accurate representation of the field soil water content. An added disadvantage of the Giddings coring method is

that it requires an expensive tractor or trailer mounted hydraulic coring machine, which may be difficult to operate in the field. Two types of driven, ring samplers were also tested [65]. These required destruction of the site because holes had to be dug to take samples at every depth. These samplers were closed at the ends causing some samples to be compacted. Calibration equation error estimates were higher with data from the ring samplers.

Evelt and Steiner [67] calibrated three Troxler and three CPN gauges in an Amarillo fine sandy loam with a sandy clay loam B horizon between 30- and 110-cm depth and a calcic horizon (Btk) below 110 cm. They used schedule 10, galvanized steel electromechanical tubing for access tubes, which were installed by pushing them into hand-augered holes of the same diameter as the outside diameter of the tube. A dry soil site was found in a fallow field and a wet site was created adjacent to it by berming an area and ponding water on it until the soil was wetted to 2-m depth. Three access tubes were installed in each site. The wetted soil was allowed to drain to field capacity (43 h) before sampling began, and sampling at the wet site was conducted in one 11-h period to minimize changes in soil water content due to drainage.

Shield counts were taken before and after counts in the access tubes, and each standard count used for calculating count ratios was the average of at least six shield counts. The CPN gauges reported a  $\chi$  ratio for each standard count. The  $\chi$  ratio is a statistic that is valuable for screening shield counts. It is the ratio of the standard deviation of counts to the square root of the mean count. Because the count of thermalized neutrons behaves as a Poisson distribution, the  $\chi$  ratio should equal unity. Shield counts for which the  $\chi$  ratio was  $<0.9$  or  $>1.1$  were eliminated from consideration. In order to avoid any influence of soil moisture on the count, shield counts were taken with the gauge resting on a depth control stand 82 cm above the soil surface. Counts in the access tubes were also taken with the gauge resting on the stand. Neutron probe readings (1-min counts) were made at 10-cm depth and in 20-cm increments below that to 190 cm.

Four soil samples were taken at each depth with a Madera sampler. For the 10-cm depth the sampler was pushed vertically into the soil until the sampling volume was centred at 10 cm, the sampler was twisted to shear the soil at the bottom and then pulled out. For depths below 10 cm, the soil was excavated on one side of the access tube and samples were taken by pushing or driving the sampler horizontally into the soil on either side of the access tube. Two samples were taken on opposite sides of the access tube just above and just below each reading depth in order, to integrate the soil volume measured by the neutron probes. During sampling, if a sample was obviously compressed or shattered it was discarded and another taken adjacent. During data reduction, the four samples were commonly averaged to give one water content per sampling depth for each access tube. However, the existence of four samples per depth for each access tube allowed samples identified as outliers during regression analysis to be discarded, particularly if values of water content and bulk density for those samples were widely divergent from the mean of the other samples.

A good range of water contents was achieved between the wet and dry sites (Fig. 7). Results of these techniques were very good. Root mean squared errors were less than  $0.012 \text{ m}^3 \text{ m}^{-3}$  for all calibration equations, and often were of the order of  $0.005 \text{ m}^3 \text{ m}^{-3}$ . There was no difference in the precision of calibration equations obtained for the two brands of moisture gauge. Enough samples were obtained to allow individual calibration equations to be calculated for the 10-cm depth, the 30- to 90-cm depth range, and the 110- to 190-cm depth range. There were important differences in the slopes and intercepts of these equations. Earlier, similar results were obtained using these calibration techniques on a Pullman clay loam and a Ulysses silt loam [44]. In the earlier study, only two access tubes were installed at each site. The Pullman soil is a Paleustoll in the US taxonomy and has a strong Bt clay horizon (illuvial clay), and a calcic horizon with up to 45% by mass of  $\text{CaCO}_3$ . Distinctly different calibration equations were found for these two horizons as well as for the 10-cm depth. In 1993, field calibrations using these methods were done on the Ulysses silt loam and the Amarillo fine sandy loam.



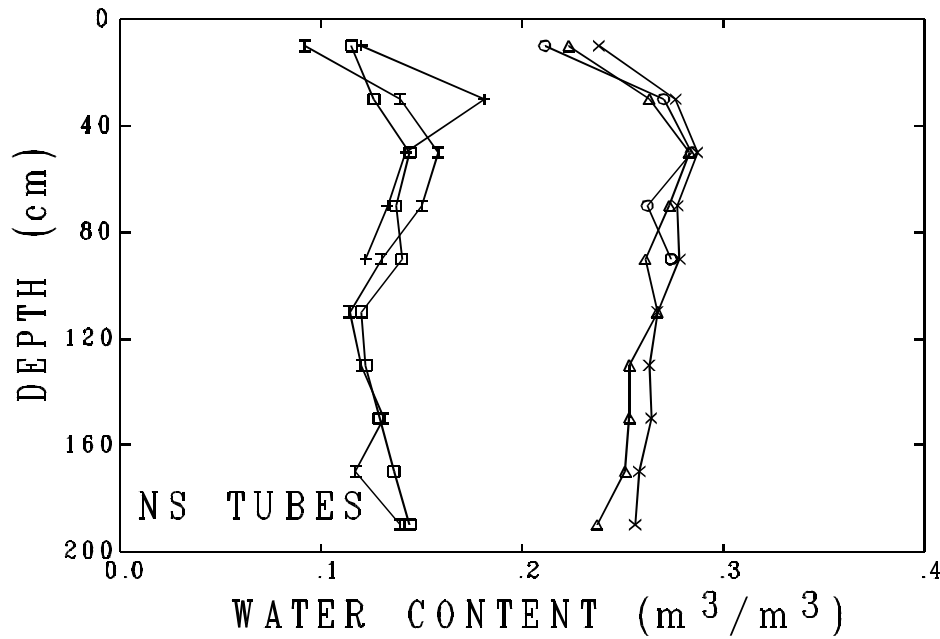


FIG. 7. Water content profiles at neutron scattering (NS) access tubes: dry site tubes: ( $\square$ ), ( $\circ$ ), and (+); and wet site tubes: (X), ( $\Delta$ ), and ( $\circ$ ).

Standard errors of estimate were less than  $0.01 \text{ m}^3 \text{ m}^{-3}$  for all horizons, and there were important differences between calibration slopes for different horizons of the Amarillo soil. For the Ulysses soil, which lacks strong illuvial clay and calcic horizons, there was no important difference between calibration equations for any depth range below the 10-cm depth.

#### 4. TEMPERATURE EFFECT ON STANDARD COUNTS

Figure 8 shows data measured over several months in 1985 using a Campbell Pacific Nuclear 503DR gauge during a field exercise at Marana, Arizona. Each day, a standard count in the shield was taken and the mean count,  $\chi$  ratio, and time were recorded. The gauge was in the field during the entire time and was equilibrated to air temperature as much as possible. A weather station in the field recorded air temperature every 15 min. The nearest 15-min average air temperature and standard counts for which  $\chi$  ratios were above 0.9 and below 1.1 were used to build the data set that is shown in the graph.

Linear regression (Fig. 8) showed that the ambient temperature explained 79% of the variation in standard count. The correlation was negative, with lower standard counts for higher temperatures. For a temperature change of  $30^\circ\text{C}$ , one could expect a change in standard count of 177. The calibration equation for this probe had a slope of  $3.59 \times 10^{-5}$ . Multiplying the slope by the change in standard count gives a change in measured water content of  $0.006 \text{ m}^3 \text{ m}^{-3}$ . This is close enough to a 1% change in water content to cause some concern. Jones and Hauser [73] measured a similar negative correlation between temperature and count rate, which corresponded to a  $0.011 \text{ m}^3 \text{ m}^{-3}$  error over a  $50^\circ\text{C}$  temperature range for the meter they used. They determined that the temperature effect was not due to the probe or the pre-amplifier circuitry in the probe, but must have been due to the counting or high voltage circuitry in the scalar unit. Hauser, using a more modern probe design, found a similar effect when all parts of the NMM were at equal temperatures [50]. But, he found a positive 6% increase in count when the probe alone was warmed from zero to  $52^\circ\text{C}$ .

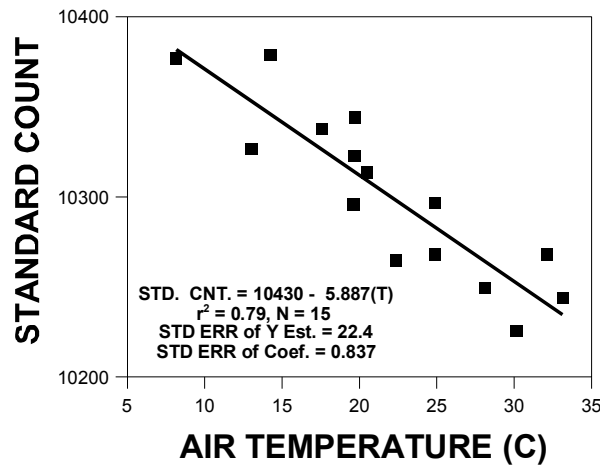


FIG. 8. Standard counts from a neutron moisture meter (model 503DR, Campbell Pacific Nuclear International, Martinez, CA) and corresponding ambient air temperatures at Marana, AZ, USA, 1985.

There are some reasons to expect that the primary source of temperature dependency is the detector tube, which contains boron trifluoride gas. Gas pressure is quite responsive to temperature changes and the detection process may be influenced by gas pressure. The counting circuitry may also be involved, particularly the high voltage and detector circuits, which are somewhat analog in nature. The rest of the circuitry in the probe would be insensitive to temperature because it is basically digital. Certainly the electronics in the gauge readout assembly, where the microcontroller is housed, are entirely digital so the problem almost certainly resides in the probe, either in the detector tube, in the pulse detection and shaping circuit, or in the high voltage circuit.

In the semiarid environment at Bushland, Texas, we may see a 17°C air temperature swing during the working day. There is some potential for the probe to be subjected to even wider temperature swings because it is used in the access tube, as well as in the shield for standard counts. We have little idea what temperature the probe is at while it is in the access tube, but we can be sure that it is changing. While travelling from one access tube to the other, the probe is locked in the shield and may equilibrate with ambient temperature. Once the probe is lowered to the bottom of the access tube it enters a much cooler or warmer environment depending on air temperature. The probe enters another temperature regime each time it is moved to a new depth stop for a reading. Because we do not have a measure of probe or detector tube temperature we cannot correct for temperature swings. We can measure the effect from standard counts in the field or using an environmental housing set to different temperatures for each standard count. But, that information is useless to us unless we can measure the probe temperature during each reading in the access tube and during each routine standard count in the field. Clearly, there is still room for improvement in modern NMM design.

## 5. COMPARISONS BETWEEN NEUTRON AND OTHER METHODS

A soil water content capacitance probe (CP) gauge (Troxler Electronic Laboratories, Inc., model SENTRY 200AP) was patterned after that of Dean et al. [74] and included some improvements while retaining the desired characteristics. Heathman [75] reported an  $r^2$  of 0.62 for a field calibration of this gauge. Evett and Steiner [67] conducted a rigorous field calibration of four of the Troxler gauges in comparison with six neutron scattering (NS) gauges, using wet and dry sites as described above. Calibrations for the CP gauges exhibited low  $r^2$  values, ranging from 0.04 to 0.71, and root mean squared error values of 0.036 to 0.058  $\text{m}^3 \text{m}^{-3}$ . Example plots illustrate the much greater scatter of CP gauge data in comparison with NS gauge data (Figs. 9 and 10).

Some possible sources of variability in the CP gauge readings can be discounted. For instance, Dean et al. [74] showed that, for their design, total thermal (0 to 30°C) and temporal (over 3 h) stability errors amounted to  $<0.005 \text{ m}^3 \text{m}^{-3}$  error in water content. They also showed that air gaps between the tube and soil would introduce large errors, thus the exacting tube installation procedure. They did not

measure the probe's sensitivity to bulk density variations. But, in a companion paper, Bell et al. [76] noted that bulk density appeared to affect the slope of calibration equations and concluded that more work was required in this area.

The CP gauge is responsive mostly to a soil layer as thin as 8 cm [76] or 12 cm [77] vertically, and within 11 cm of the probe centreline [77]. Thus, small-scale variations in soil properties are more likely to influence the probe's readings than would be the case for the NS gauge. The electric field induced in the soil by the CP is influenced by boundaries between soil volumes having different permittivities [74]. Thus, bulk density or  $\theta_v$  variations on a small scale could set up boundaries that would influence the size and shape of the sampled volume. Boot and Watson [78] noted that sample heterogeneities can cause anomalous readings from capacitance probes applied to building materials, especially when the wavelength approaches the scale of heterogeneity. Wobschall [79] pointed out that heterogeneous soils can also cause poor results.

Another possible explanation for the poor results with the CP gauges is that the measurement volume is considerably smaller than reported by Bell et al. [76] and Troxler Electronic Laboratories [77]. If this were so then the soil sampling method used by Evett and Steiner [67] would be inappropriate. However, the 15.24-cm measurement interval provided by the stops on the CP gauge probe handle would be too large if the sampling volume were smaller than that stated by Troxler Electronic Laboratories [77]. If the sampling volume is indeed much smaller than reported, then the use of the CP gauge must be reevaluated because many more samples at much smaller vertical sampling intervals must be taken to provide accurate integration of the soil water content profile. In fact, if this hypothesis is true it may be difficult to accurately portray the soil water content profile in many soils because the representative elemental volume may be larger than the gauge's sampling volume. Field calibration of this gauge would also be problematic in this case because an exacting relationship between probe position in the tube and position of soil sampling is implied.

Tomer and Anderson [80] obtained better results with the Troxler CP gauge in a comparison with an NS gauge in a deep aeolian sand (Zimmerman fine sand). Samples for calibration were obtained by taking 5-cm diameter vertical cores. Access tubes were then installed in the coring holes. Because the sand was not cohesive, bulk density values were not used from these samples, but bulk densities from a previous study were used to calculate volumetric water contents. The NS gauges calibration resulted in an  $r^2$  value of 0.966 ( $N = 31$ ). The CP gauge calibration gave an  $r^2$  value of 0.888 ( $N = 73$ ), and was similar to the manufacturer's calibration equation, a fact that is not surprising given that the manufacturer calibrates in sand. Soil water lost in a 1.5-m profile over 2 weeks averaged 1.2 cm less as measured by the CP gauge compared with the NS gauge, and the CP gauge routinely gave higher water content measurements. The CP gauge had much higher spatial resolution, a fact that rendered it susceptible to problems with access tube installation.

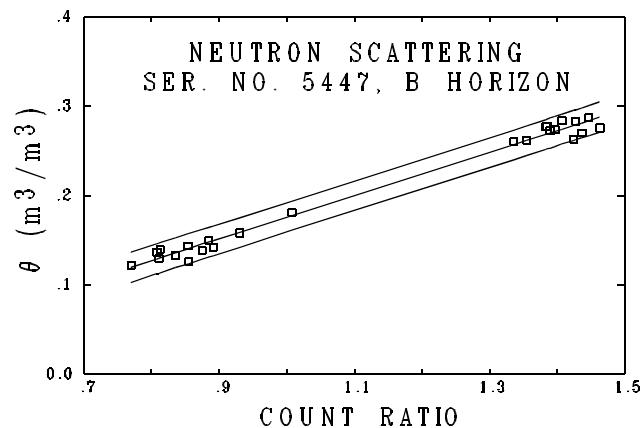


FIG. 9. Typical volumetric water content ( $\theta$ ) vs. count ratio relationship in the B horizon (tubes 1–6). Middle line is the regression line, upper and lower lines are 95% confidence limits on the predictions.

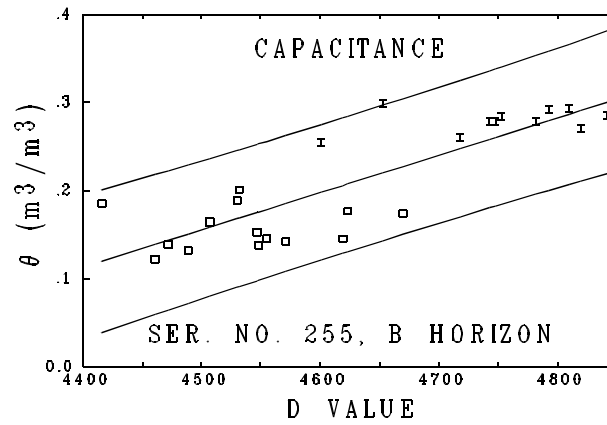


FIG. 10. Typical relationship between volumetric water content ( $\theta$ ) and the absolute value of the measured frequency shift ( $D$ ) from capacitance gauges (tubes 7–13), showing dry site data ( $\square$ ) and wet site data ( $\bullet$ ). Middle line is the regression line, upper and lower lines are 95% confidence limits on predictions.

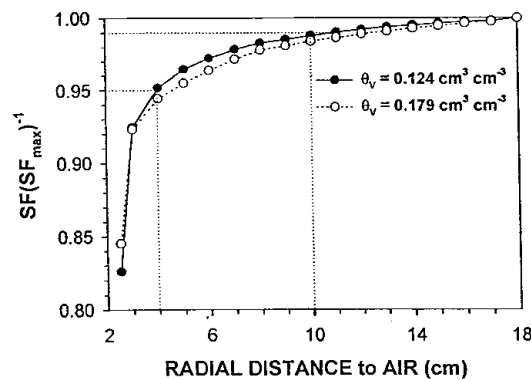


FIG. 11. Relative radial sensitivity of EnviroScan sensors as a function of radial thickness of soil around the access tube (from [82]).

Mohamed et al. [81] compared the Humicap (Nardeux, Loches, France) capacitance probe to a neutron probe (Solo 25, Nardeux, Loches, France). The capacitance probes were buried in augered holes with direct contact between the electrodes and the soil. The capacitance probes were “highly sensitive to change in soil structure and texture,” but provided better accuracy than the neutron probe, which was calibrated by a theoretical method. It is likely that the better results obtained for capacitance probes in this study were due to the lack of an air gap between the electrodes and the soil.

Paltineanu and Starr [82] calibrated a capacitance probe (EnvironSCAN, Sentek Pty Ltd., South Australia) in the laboratory using a silt loam soil with good results ( $r^2 = 0.992$ ,  $N = 15$ ,  $\theta_v$  range =  $0.07 - 0.37 \text{ m}^3 \text{ m}^{-3}$ ,  $\text{RMSE} = 0.009 \text{ m}^3 \text{ m}^{-3}$ ). Boxes were packed very uniformly (CV for bulk density = 0.5 to 2.9%, CV for  $\theta_v = 0.0054$  to 0.065%) with soil at four different water contents for the calibration. The extreme uniformity of packing brings into question how appropriate the calibration would be for a field soil, which is likely to be much less uniform in bulk density and water content on a small scale. Tests of radial sensitivity showed that 99% of the sensitivity was within a 10-cm radius outside of the access tube, and 92% of the sensitivity was within a 3-cm radius of soil outside the access tube (Fig. 11). This reveals that the probe will be quite sensitive to small scale variations of soil properties close to the access tube. Later, the same authors [83] installed these probes in the field for long-term measurements of profile water content. Though they reported success, they did not test to determine if the laboratory calibration proved accurate in the field. The tests they did conduct were comparisons with crop water use estimated using an atmometer, and cannot be considered rigorous. Oddly, they did not report any water contents, only soil water storage and change in storage data. Paltineanu and Starr

[82] considered it inappropriate to compare the capacitance method with neutron thermalization due to differences in measurement method and sphere of influence. However, such differences might well be the point of a comparison, as was shown by Evett and Steiner [67].

At this writing (2000), many capacitance type soil moisture probes or gauges are being introduced in the marketplace. Some of these respond quite well to the dynamics of soil water content, including that due to plant water uptake. Demonstrations have shown that the dynamic behaviour of plant water uptake can provide important information needed for irrigation scheduling. But, there is a lack of scientific literature supporting claims of accuracy of soil water content measurement with these devices, demonstrating that laboratory calibrations may be used successfully in the field, or demonstrating successful field calibrations. Capacitance probes that employ sensors in a plastic access tube are the closest analogue of the neutron probe deployed in an access tube. However, studies to date show that capacitance probes have a very narrow radial range of sensitivity outside of the access tube and thus suffer from disadvantages that include

- sensitivity to soil disturbance during tube installation, and
- sensitivity to small scale variations in soil bulk density (including macroporosity), water content, and texture, which are common to many soils.

Other studies have shown that capacitance probes are still sensitive to soil salinity, temperature, and texture, though perhaps less so than in the past. Though it may be useful for some irrigation scheduling needs, the capacitance probe still cannot be considered a replacement for the neutron probe for soil water content measurements for which accuracy is important.

## REFERENCES

- [1] PIEPER, G.F., The Measurement of the Moisture Content of Soil by the Slowing of Neutrons, Thesis, Cornell University, Ithaca (1949).
- [2] BELCHER, D.J., et al., Technical Development Report 127, Technical Development and Evaluation Center, Civil Aeronautics Administration, Indianapolis (1950).
- [3] GARDNER, W., KIRKHAM, D., Determination of soil moisture by neutron scattering, *Soil Sci.* **73** (1952) 391–401.
- [4] NEVILLE, O.K., VAN ZELST, T.W., “Design and application of the Nuclear-Chicago D/M-Gauge”, Symposium on Nuclear Methods for Measuring Soil Density and Moisture, ASTM Special Technical Publication No. 293, American Society for Testing Materials, Philadelphia (1960) 3–13.
- [5] HAUSER, V.L., Estimates of Precision of Moisture Measurements Made with the d/M Gage Neutron Moisture Meter During 1959, Unpublished Research Report of the USDA-ARS, Western Soil and Water Management Research Branch, Southwestern Great Plains Field Station, Bushland, TX (1959).
- [6] VAN BAVEL, C.H.M., et al., Soil moisture measurement by neutron moderation, *Soil Sci.* **82** (1956) 29–41.
- [7] OLGAARD, P.L., On the Theory of the Neutron Method for Measuring the Water Content in Soil, Riso Report No 97, Danish Atomic Energy Commission, Roskilde (1965).
- [8] STONE, J.F., “Neutron physics considerations in moisture probe design”, *Irrigation and Drainage* (HARRIS, S.R., Ed.), Am. Soc. Civil Engr., New York (1990) 1–8.
- [9] VAN BAVEL, C.H.M., et al., Calibration and characteristics of two neutron moisture probes, *Soil Sci. Soc. Am. Proc.* **25** (1961) 329–334.
- [10] NAKAYAMA, F.S., ALLEN, S.G., “Application of neutron soil surface water monitoring for plant establishment”, *Irrigation and Drainage* (HARRIS, S.R., Ed.), Am. Soc. Civil Engr., New York (1990) 210–217.
- [11] SHALHEVET, J., BIELORAI, H., Crop water requirement in relation to climate and soil, *Soil Sci.* **125** (1978) 240–247.
- [12] SHALHEVET, J., et al., *Irrigation of Field and Orchard Crops Under Semi-arid Conditions* International Irrigation Information Centre, Volcani Centre, Bet Dagan (1976).
- [13] ERIE, L.J., et al., *Consumptive Use of Water by Crops in Arizona*, US Water Conservation Laboratory, Phoenix (1965).

- [14] ERIE, L.J., et al., Consumptive Use of Water by Major Crops in the Southwestern United States, USDA-ARS, Conservation Research Report No. 29 (1982).
- [15] Vegetative Water Used Studies 1954–1960, Bulletin No. 113, Interim Report of the California Dept. of Water Resources (1963).
- [16] JENSEN, M.E., Evaluating irrigation efficiency, J. Irrig. Drainage Div. ASCE **93**, no. **IR1** (1967) 83–98.
- [17] VACHAUD, G.C., et al., Method de caracterisation hydrodynamique d'un sol sature. Applications a deuz types de sol du Senegal en vue de la determination des termes du bilan hydrique, Ann. Agron. **29** (1978) 1–36.
- [18] HOWELL, T.A., et al., “History of lysimeter design and use for evapotranspiration measurements”, Lysimeters for Evapotranspiration and Environmental Measurements, ASCE, New York (1991) 1–9.
- [19] GREBET, R., CUENCA, R.H., “History of lysimeter design and effects of environmental disturbances”, Lysimeters for Evapotranspiration and Environmental Measurements, Am. Soc. Civil Eng., New York (1991) 10–18.
- [20] BLACK, T.A., et al., Hydraulic load cell lysimeter, construction, calibration and tests, Soil Sci. Soc. Am. Proc. **32** (1968) 623–629.
- [21] DUGAS, W.A., BLAND, W.L., Springtime soil temperatures in lysimeters in Central Texas, Soil Sci. **152** (1991) 87–91.
- [22] LOURENCE, F., MOORE, R., “Prefabricated weighing lysimeter for remote research stations”, Lysimeters for Evapotranspiration and Environmental Measurements, Am. Soc. Civil Eng. (1991) 432–439.
- [23] MAREK, T.H., et al., Design and construction of large weighing monolithic lysimeters, Trans. ASAE **31** (1988) 477–484.
- [24] VAN BAVEL, C.H.M., STIRK, G.B., Soil water measurement with and  $\text{Am}^{241}$ -Be neutron source and an application to evaporimetry, J. Hydrol. **5** (1967) 40–46.
- [25] CUENCA, R.H., Model for evapotranspiration using neutron probe data, J. Irrig. Drain. Engrg. ASCE **114** (1988) 644–663.
- [26] WRIGHT, J.L., “Comparison of ET measured with neutron moisture meters and weighing lysimeters”, Irrigation and Drainage (HARRIS, S.R., Ed.), Am. Soc. Civil Eng., New York (1990) 202–209.
- [27] CARRIJO, O.A., CUENCA, R.H., Precision of evapotranspiration estimates using neutron probe, J. Irrig. Drain. Engrg. ASCE **118** (1992) 943–953.
- [28] BERTUZZI, P., et al., Sampling strategies for soil water content to estimate evapotranspiration, Irrig. Sci. **14** (1994) 105–115.
- [29] EVETT, S.R., et al., “Evapotranspiration by soil water balance using TDR and neutron scattering”, Management of Irrigation and Drainage Systems, Am. Soc. Civil Eng., New York (1993) 914–921.
- [30] HAISE, H.R., HAGAN, R.M., “Soil, plant, and evaporative measurements as criteria for scheduling irrigation”, Irrigation of Agricultural Lands, (HAGAN, R.M., et al., Eds.), ASA, Madison (1967) 577–604.
- [31] GEAR, R.D., et al., Irrigation scheduling with neutron probe, J. Irrig. Drain. Div. ASCE **103** (1977) 291–298.
- [32] CAMPBELL, G.S., CAMPBELL, M.D., “Irrigation scheduling using soil moisture measurements: Theory and practice”, Advances in Irrigation, Vol. 1, (HILLEL, D., Ed.), Academic Press, New York (1982) 25–42.
- [33] INTERNATIONAL ATOMIC ENERGY AGENCY, Comparison of Soil Water Measurement Using the Neutron Scattering, Time Domain Reflectometry and Capacitance Methods, IAEA-TECDOC-1137, Vienna (2000).
- [34] GARDNER, W.H., CALISSENDORFF, C., “Gamma ray and neutron attenuation measurement of soil bulk density and water content”, Isotope and Radiation Techniques (Proc. Symp. Techniques in Soil Physics and Irrigation Studies, Istanbul), IAEA, Vienna (1967) 101–113.
- [35] GARDNER, W.H., “Water content”, Methods of Soil Analysis, Part 1, Second Edition (KLUTE, A., Ed.), ASA and SSSA, Madison (1986) 493–544.
- [36] GREACEN, E.L., Soil Water Assessment by the Neutron Method, CSIRO, Melbourne (1981).

- [37] PARKES, M.E., SIAM, N., Error associated with measurement of soil moisture change by neutron probe, *J. Agric. Engng. Res.* **24** (1979) 87–93.
- [38] HIGNETT, C., EVETT, S.R. “Neutron thermalization”, *Methods of Soil Analysis*, Third Edition. ASA-CSSA-SSSA, Madison (2000).
- [39] HODNETT, M.G., BELL, J.P., Neutron probe standards: Transport shields or a large drum of water? *Soil Sci.* **151** (1991) 113–120.
- [40] DICKEY, G.L., “Factors affecting neutron gauge calibration”, *Irrigation and Drainage* (Harris, S.R., Ed.), Am. Soc. Civil Engr., New York (1990) 9–20.
- [41] ALLEN, R.G., SEGURA, D., “Access tube characteristics and neutron meter calibration”, *Irrigation and Drainage* (Harris S.R., Ed.) Am. Soc. Civil Engr., New York (1990) 21–31.
- [42] STONE, J.F., NOFZIGER, D.L., Calibration of neutron moisture probes by transfer through laboratory media: I. Principles, *Soil Sci.* **160** (1995) 155–163.
- [43] STONE, J.F., et al., Calibration of neutron moisture probes by transfer through laboratory media: II. Stability experience, *Soil Sci.* **160** (1995) 164–175.
- [44] EVETT, S.R. “Some aspects of time domain reflectometry (TDR), neutron scattering, and capacitance methods of soil water content measurement”, *Comparison of Soil Water Measurement Using the Neutron Scattering, Time Domain Reflectometry and Capacitance Methods*, IAEA-TECDOC-1137, Vienna (2000) 5–49.
- [45] ALLEN, R.G., et al., “Effect of moisture and bulk density sampling on neutron moisture gauge calibration”, *Management of Irrigation and Drainage Systems, Integrated Perspectives* (Allen, R.G., Neale, C.M.U., Eds.), Am. Soc. Civil Engr., New York (1993) 1145–1152.
- [46] HANSON, B.R., DICKEY, G.L., Field Practices Affect Neutron Moisture Meter Accuracy, *California Agriculture* **Nov.–Dec.** (1993) 29–31.
- [47] NAKAYAMA, F.S., REGINATO, R.J., Simplifying neutron moisture meter calibration, *Soil Sci.* **133** (1982) 48–52.
- [48] STONE, J.F., et al., “Performance factors of neutron moisture probes related to position of source on detector”, *Management of Irrigation and Drainage Systems, Integrated Perspectives* (ALLEN, R.G., NEALE, C.M.U., Eds.), Am. Soc. Civil Engr., New York (1993) 1128–1135.
- [49] GRANT, D.R. Measurement of soil moisture near the surface using a moisture meter, *J Soil Sci.* **26** (1975) 124–129.
- [50] HAUSER, V.L. Neutron meter calibration and error control, *Trans. ASAE* **27** (1984) 722–728.
- [51] VANDERVAERE, J.P., et al., Error analysis in estimating soil water balance of irrigated fields during the EFEDA experiment: 1. Local standpoint, *J. Hydrol.* **156** (1994) 351–370.
- [52] VAN BAVEL, C.H.M., *Measurement of Soil Moisture Content by the Neutron Method*, USDA-ARS (1958).
- [53] McHENRY, J.R., Theory and application of neutron scattering in the measurement of soil moisture, *Soil Sci.* **95** (1963) 293–307.
- [54] LAWLESS, G.P., et al., Soil moisture interface effects upon readings of neutron moisture probe, *Soil Sci. Soc. Amer. Proc.* **27** (1963) 502–507.
- [55] STONE, J.F., WEEKS, D.L., Precision of evapotranspiration estimates using neutron probe. Discussion, *J. Irrig. Drain. Engng.* **120** (1994) 989–991.
- [56] HAVERKAMP, R., et al., Error analysis in estimating soil water content from neutron probe measurements: 1. Local standpoint, *Soil Sci.* **137** (1984) 78–90.
- [57] SINCLAIR, D.F., WILLIAMS, J., Components of variance involved in estimating soil water content and water content change using a neutron moisture meter, *Aust. J. Soil Res.* **27** (1979) 237–247.
- [58] VANDERVAERE, J.P., et al., Error analysis in estimating soil water balance of irrigated fields during the EFEDA experiment: 2. Spatial standpoint. *J. Hydrol.* **156** (1994) 371–388.
- [59] VAUCLIN, M., et al., Error analysis in estimating soil water content from neutron probe measurements: 2. Spatial standpoint. *Soil Sci.* **137** (1984) 141–148.
- [60] SARTZ, R.S., CURTIS, W.R., *Field Calibration of a Neutron-Scattering Soil Moisture Meter* USDA-Forest Service, Lake States Forest Experiment Station (1961).
- [61] RAWLS, W.J., ASMUSSEN, L.E., Neutron probe field calibration for soils in the Georgia coastal plain, *Soil Sci.* **116** (1973) 262–265.
- [62] DICKEY, G.L. “Field calibration of neutron gauges: SCS method”, *Irrigation and Drainage* (Harris, S.R., Ed.), Am. Soc. Civil Engr., New York (1990) 192–201.

- [63] MORRIS, P.H., et al., Engineering Implications of the Linear Regression of Geotechnical Data, *Aust. Civil Eng. Trans.*, **4** (1987) 239–247.
- [64] AMERICAN SOCIETY FOR TESTING AND MATERIALS, “Tentative method for thin-walled sampling of soils”, *Procedures for Testing Soils*, Fourth Edition, Astm, Philadelphia (1964) 53–56.
- [65] DICKEY, G.L., et al., “Soil bulk density sampling for neutron gauge calibration”, *Management of Irrigation and Drainage Systems, Integrated Perspectives* (ALLEN, R.G., NEALE, C.M.U., Eds.) *Am. Soc. Civil Engr.*, New York (1993). 1103–1111
- [66] DICKEY, G.L. Radiation Safety, Calibration and Use of the Neutron Soil Moisture Gauge, Second Draft, USDA-SCS, Midwest National Technical Center, Lincoln (1982).
- [67] EVETT, S.R., STEINER, J.L., Precision of neutron scattering and capacitance type moisture gages based on field calibration, *Soil Sci. Soc. Am. J.* **59** (1995) 961–968.
- [68] REGINATO, R.J., NAKAYAMA, F.S., Neutron probe calibration based on plastic transfer standards, *Soil Sci.* **145** (1988) 381–384.
- [69] McCAULEY, G.N., STONE, J.F., Source-detector geometry effect on neutron probe calibration, *Soil Sci. Soc. Am. Proc.* **36** (1972) 246–250.
- [70] McGUINNESS, J.L., et al., Soil moisture measurements with the neutron method supplement weighing lysimeters, *Soil Sci. Soc. Am. Proc.* **25** (1961) 339–342.
- [71] STONE, J.F., et al., The ASCE neutron probe calibration study: Overview. ”, *Management of Irrigation and Drainage Systems, Integrated Perspectives* (ALLEN, R.G., NEALE, C.M.U., Eds.), *Am. Soc. Civil Engr.*, New York (1993) 1095–1102.
- [72] DICKEY, G.L., et al., Neutron gauge calibration comparison of methods”, *Management of Irrigation and Drainage Systems, Integrated Perspectives* (ALLEN, R.G., NEALE, C.M.U., Eds.), *Am. Soc. Civil Engr.*, New York (1993). 1136–1144.
- [73] JONES, O.R., HAUSER, V.L., Results of Neutron Moisture Meter Test to Evaluate: 1. Effect of temperature on count rate, 2. Probe storage in dry sand, and 3. Three timing methods, USDA-ARS, Research report no. 358, Southwester Great Plains Field Station, Bushland, TX (1962).
- [74] DEAN, T.J., et al., Soil moisture measurement by an improved capacitance technique: Part I. Sensor design and performance, *J. Hydrol. (Amsterdam)* **93** (1987) 67–78.
- [75] HEATHMAN, G.C., Soil Moisture Determination Using a Resonant Frequency Capacitance Probe, Paper no. 931053, American Society of Agricultural Engineers, St. Joseph, MI (1993).
- [76] BELL, J.P., et al., Soil moisture measurement by an improved capacitance technique: Part II. Field techniques, evaluation and calibration, *J. Hydrol. (Amsterdam)* **93** (1987) 79–90.
- [77] TROXLER ELECTRONIC LABORATORIES. Troxler Technical Brief: Comparing the Sentry 200-AP and the Model 4300 Moisture Probes, Troxler Electronic Lab., Research Triangle Park, NC (1993).
- [78] BOOT, A.R., WATSON, A., “Applications of centrimetric radio waves in nondestructive testing,” *ASTM-RILEM Symp. Appl. Adv. Nucl. Phys. Testing Materials*, Texas A&M University, College Station (1964) 3–24.
- [79] WOBSCALL, D., A frequency shift dielectric soil moisture sensor, *IEEE Trans. Geosci. Electron.* **GE-16** (1978) 112–118.
- [80] TOMER, M.D., ANDERSON, J.L., Field evaluation of a soil water-capacitance probe in a fine sand, *Soil Sci.* **159** (1995) 90–98.
- [81] MOHAMED, S.O., et al., Field evaluation and error analysis of soil water content measurement using the capacitance probe method, *Soil Sci. Soc. Am. J.* **61** (1997) 399–408.
- [82] PALTINEANU, I.C., STARR, J.L., Real-time soil water dynamics using multisensor capacitance probes: Laboratory calibration, *Soil Sci. Soc. Am. J.* **61** (1997) 1576–1585.
- [83] STARR, J.L., PALTINEANU, I.C., Soil water dynamics using multisensor capacitance probes in nontraffic interrows of corn, *Soil Sci. Soc. Am. J.* **62** (1998) 114–122.



## WATER USE OF CEREAL-CANOLA-LUCERNE ROTATIONS IN SOUTHEASTERN AUSTRALIA

C.J. SMITH, W.J. BOND, K. VERBURG  
CSIRO Land and Water,  
Canberra

F.X. DUNIN  
CSIRO Plant Industry,  
Floreat Park, Western Australia

Australia

### Abstract

The water balances of several annual crops grown in rotation with lucerne were monitored and compared with that under a native white-cypress forest near Wagga Wagga, New South Wales. Water use by the white cypress forest was slightly greater than that by canola, but much less than by cereals and lucerne during the growing season. The cereal and canola crops have a cyclic water use pattern (high in spring, low in summer/autumn) while that for the white cypress is more uniform through the year. Lucerne behaves more like the cypress forest. It uses water throughout the year, and is a high water use alternative to be grown in rotation with crops. It extracts water below the 'nominal' rooting depth of annual crops that has built up from several years of cropping. This reduces the likelihood of deep drainage in the subsequent winter by creating a buffer for water storage. Simulations using APSIM (Agricultural Production Systems Simulator) run on historical weather data (1957 to 1997) confirmed the potential of lucerne to keep the soil drier and reduce drainage in a rotation. Soil water storage gradually increased under wheat after the lucerne was removed, and was similar to that under continuous wheat after 2 to 3 years. The refilling of the buffer depends on the timing and amount of rainfall. In general, this means that the wheat phase should probably not exceed 2 to 3 years. Furthermore, the drying under lucerne happens rapidly and often within the first summer/autumn of the lucerne phase, with little difference in water use between lucerne and wheat during winter/spring. These results suggest that practical benefits can be obtained by growing lucerne for as little as 12 months, yet, at other times, it may need to be grown for 2 or even 3 years.

### 1. INTRODUCTION

Many farming systems in temperate Australia drain more water beyond the root zone than do natural ecosystems. This is a problem because groundwater moves slowly in the Australian landscape and the change from native vegetation to annual cropping has been associated with rising water tables and the mobilization of salt stored over many thousands of years. It is ironic that, in Australia, both water and nutrients limit agriculture, yet it is the loss of both beyond the root-zone of annual crops and pastures that is the fundamental cause of salinity and acidification [1].

Rainfed crops in temperate Australia are mainly annual species for food and fibre production. They are grown in the cooler periods with adequate soil water, as are annual pastures. They show high rates of water use from a root zone that is necessarily restricted in depth. In contrast, the preceding natural ecosystems were dominated by perennial woody and herbaceous species. These systems used water sparingly in the cool seasons when soil water was plentiful. Thus, soil water was conserved in a deep root zone, ensuring survival over the dry summer months [1]. Peck and Hurle [2] estimated a difference of ~ 50 mm/year in long-term recharge rates between natural and agricultural communities under the same rainfall regime. However, there is considerable uncertainty in the drainage estimates, which are often <10% of annual rainfall.

A lack of quantitative information on drainage rates under natural and agricultural systems is hindering management decisions to control landscape degradation. Detailed measurements of the water balance combined with mechanistic models are needed to develop new farming systems that will arrest the degradation. Potential solutions must be evaluated as part of the larger-scale ecological and hydrological processes that operate over the landscape and regional basins. The challenge facing

dryland agriculture is to develop new farming systems that are productive and have a low drainage term in the water balance. This paper presents experimental data and simulations of water use of an annual crop-lucerne rotation and a native white-cypress forest.

## 2. MATERIALS AND METHODS

The water balances of cropping systems in five paddocks were monitored and compared with those at two sites in a native white-cypress forest. The forest was chosen to allow evaluation of the water balance of native vegetation in the same region as the cropping systems. Measurement and modelling approaches were used to investigate the water balances and to extrapolate measurements made over several years to longer-time periods, thereby incorporating year-to-year variability into the analysis. In one paddock, two weighing lysimeters (1.8-m deep) were used to estimate evapotranspiration ( $Et$ ) [3]. At other sites, water balance measurements were limited to rainfall and changes in soil water contents, although Bowen-Ratio equipment was used to measure  $Et$  for selected periods. Crop sequences included annual winter cereals (wheat or triticale), canola, and lucerne (Table I). The two forest sites differed in tree density and age; one was dominated by a high density of regenerating saplings while the other was an open stand of mature trees.

Automatic weather stations were installed at each site to measure rainfall, solar and net radiation, air temperature, relative humidity, and wind speed. Soil water content and storage were measured using a neutron moisture meter in access tubes installed to 3-m depth (four locations per paddock) under the agricultural crops and to 6-m depth (three locations at each site) in the cypress forest. Soil water measurements to a depth of 3 or 6 m were commenced at all sites in March 1999. Additional access tubes were installed in April 2000 in the paddock sown to lucerne in July 1999, because the depth of water extraction had reached 3 m. Water content measurements were disrupted each autumn in cropped paddocks because of the need to remove and reinstall the 1-m deep access tubes, and to remove the top (0.2 m) of the deeper tubes to permit cultivation and sowing.

If certain conditions are satisfied,  $Et$  can be calculated from the water balance:

$$Et = P - \Delta S - D - RO \quad (1)$$

where

$P$  is rainfall, (mm)

$\Delta S$  is the increase in water storage in the soil (to depth  $Z$ ) (mm),

$D$  is drainage past the depth  $Z$  (mm),

and  $RO$  is surface runoff (mm).

TABLE I. CROPPING SEQUENCE FOR THE PADDOCKS (1997–2000)

Site	No.	1997	1998	1999	2000
Charles Sturt	1	Lucerne	Canola	Triticale	Lupin
University	2	Canola	Triticale	Lucerne	Lucerne
Wagga Wagga					
Harts (Juee)	1	Field Peas	Canola/lucerne <sup>a</sup>	Lucerne	Lucerne
	2	Canola	Wheat	Canola	Wheat
Munros	1	Lucerne/pasture	Canola	Wheat	Barley/lucerne <sup>a</sup>
(Coolamon)					

<sup>a</sup>Lucerne undersown.

Equation (1) assumes that the depth of soil water extraction by vegetation is less than  $Z$ . It can be simplified by assuming that runoff is zero and drainage past the deepest measurement depth ( $Z = 3$  m for crops, 5 m for the forest) was negligible. For these conditions,

$$Et = P - \Delta S \quad (2)$$

Equation (2) was used to calculate  $Et$  at all sites from measurements of rainfall and the change in soil water storage.

Water fluxes past certain depths in the soil profile can be calculated from soil water content measurements provided that they extend beyond the root-zone, and that the soil below the root zone is relatively dry so that there is no drainage past the deepest measurement depth. Deep drainage past the root zone of annual crops could, therefore, be determined when these conditions were met. Once the soil at depth became wet and there was the possibility of water movement past the maximum measurement depth, this method was no longer applicable. Under these circumstances, estimates of  $Et$  (e.g. from lysimeters or Bowen Ratio) were used to estimate deep drainage from the water balance.

### 3. RESULTS AND DISCUSSION

Cumulative  $Et$  between water content measurement dates, calculated from Eq. (2), agreed with those obtained from the two lysimeters [paddock #1, Charles Sturt University (CSU)] for the period 16 June 1999 to 29 August 2000, and that determined with the Bowen Ratio method for 15 August to 10 November 1999 in the lucerne paddock at Harts (Fig. 1). These data indicate that the assumptions required for Eq. (2) to be appropriate were valid for these paddocks over this period. Furthermore, during 1999/2000, the soil below 2 m was relatively dry throughout the year in most paddocks and changes in soil moisture below 2 m were negligible during the growing season. Therefore, the use of Eq. (2) to calculate  $Et$  for all sites would appear to be valid with the exception of the lucerne undersown in 1998. For this crop, there was evidence that water extraction was occurring from depths greater than 3 m by the 4 November 1999, after which  $Et$  calculated from Eq. (2) is likely to have underestimated the water use of the lucerne.

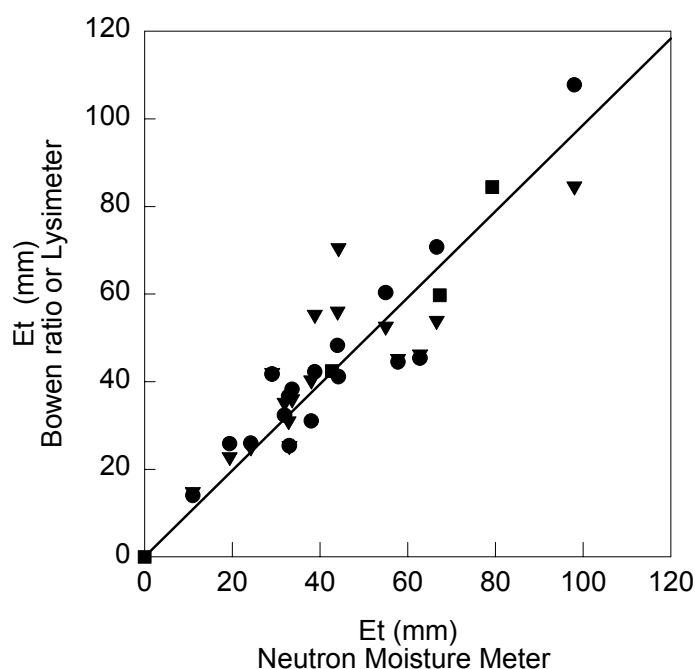


FIG. 1. Comparison of  $Et$  measured with the lysimeters (north ▼ and south ●) or the Bowen Ratio method (■) and that estimated with the neutron moisture meter [Eq. (2)] for triticale, fallow and lupin, or lucerne undersown in 1998. The line of best fit is  $Y = 0.98X$  ( $R^2 = 0.80$ ).

Cumulative Et for all the crops during the 1999 growing season (the only complete season for which measurements are available) are shown as a function of cumulative rainfall in Fig. 2. This method of presentation was chosen because water use is affected not only by the type of crop, but also by the available rainfall, which varies across the Wagga Wagga region. Biomass of the crops for this period are included in Fig. 2. In general, Et increased with increasing rainfall. Of the three annual crops grown in 1999, only canola used less water than seasonal rainfall (23 March – 13 December 1999). Second-year lucerne (sown in 1998) used more water than rainfall, whereas the lucerne planted in July 7 1999 was still getting established and its water use was about the same as rainfall. Wheat had the highest cumulative Et, and the highest excess of Et over rainfall. This was possibly because it followed a canola crop, which tends to leave more plant-available water behind in the soil profile than do other crops. It has also been suggested that the rooting system of canola improves soil structure, which assists water extraction by the subsequent crop [4].

Of the crops and lucerne, canola used the least water—less water than rainfall—resulting in a net storage of water in the soil profile. The lower water use by canola during the period from sowing to harvest of the cereal crops is consistent with our previous finding for the 1998 growing season. Furthermore, it is also consistent with data reported by Kirkegaard et al. [4] that showed a net increase in soil water storage of from 12 to 124 mm after canola. The lower water use is attributed to the different growth pattern compared with cereal crops. After flowering (mid-September), the effective leaf area of canola rapidly declines to about 1. At this time, it is likely to use less water than cereal crops which have leaf area indices >3 and use water at high rates. Furthermore, canola is generally ready for windrowing about a month earlier than the harvest date of cereal crops. Canola crops, therefore, do not have the capacity to use rain that falls late in the growing season, which, in the Wagga Wagga region, averages about 6% of annual precipitation. In contrast, cereals use much of this rain, resulting in a drier profile.

Water use in the forest was less than rainfall, for the period shown (Fig. 2). However, after a full 12 months, water use in the forest was slightly greater than rainfall. Three forest data points are presented because changes in soil water content at one of the neutron access tubes had different patterns and magnitude compared with the other two tubes at the open site. The difference seems to have been related to surrounding vegetation; two tubes were more in the open, whereas the other tube was adjacent to a large eucalypt and several cypress trees.

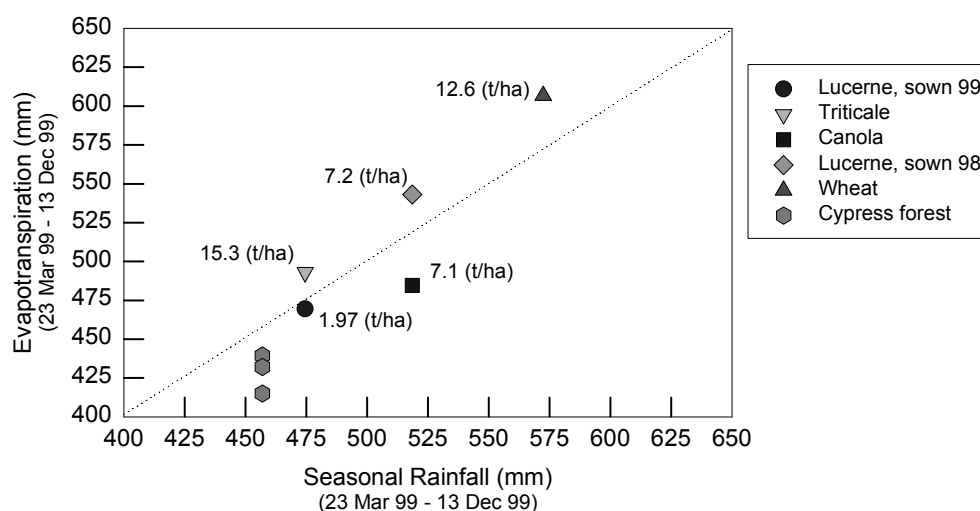


FIG. 2. Cumulative Et (23 March 99 – 13 December 99) for crops and the cypress forest (Lester) in the Wagga Wagga region. The broken line represents 1:1. Numbers are the total aboveground biomass of the crops produced during this period.

Our data allow estimates of water storage and use at various depths in the soil profile, and of water movement past the effective rooting depth of the annual crops. None of the annual crops extracted significant amounts of water from deeper than 1.2 m, which is assumed to be the bottom of the root zone. With no deep-rooted agricultural plants (e.g. lucerne) included in the rotation, water moving past 1.2 m is lost to the vegetation and may eventually become recharge for groundwater. Drainage past the root zone of 22 and 14 mm occurred beneath the triticale and wheat, respectively, during the 12 months (23 March 1999 – 3 April 2000). It occurred during the growing season, mainly between the 23 March and 23 June 1999, during which time the crops were establishing. No drainage occurred beneath the 1999 canola crop; this is attributed to the high yielding wheat crop (6 t/ha) in the previous year (1998), which is considered to have effectively dried the upper part of the soil profile. In addition, the canola re-sprouted after harvest and vigorously used water during the summer (1999/2000).

The two lucerne pastures both mined significant amounts of water from below the root zone of the annual crops over this 12-month period, creating a buffer that will allow some drainage past the root zone to be tolerated when the paddocks are returned to annual cropping. Although there was no net drainage past 1.2 m for this period, 27 mm drainage occurred under the lucerne sown in July 1999 (CSU paddock #2) during the annual crop-growing season (March – December 1999). It occurred when the lucerne was establishing and the paddock was essentially bare.

Annual drainage (March 1999 to April 2000) past 1.2 m at the forest sites was approximately zero. There was significant water movement past 1.2 m during March to December 1999 at the sites dominated by large trees, but this water was extracted again in the summer months.

Lucerne behaved more like native vegetation and used water throughout the year. It is a high water use alternative, grown in rotation with annual crops because it effectively scavenges plant-available water from below 1.2 m, and can root to a depth of at least 3 m in this environment and on these soils. Extraction of soil water below the “nominal” rooting depth of annual crops, built up from several years of cropping, reduces the likelihood of deep drainage in the subsequent winter by creating a buffer for water storage.

Although we have no soil water content measurement prior to March 1999, Et for the sequence of crops grown since 1997 in paddock #1 at CSU was measured using the weighing lysimeters (Fig. 3). There was net accumulation of 39 mm water in the soil during the nominal growing season (8 May 1997 – 1 December 1997) as evidenced by rainfall exceeding Et of the lucerne. In preparation for cropping, the lucerne was sprayed with glyphosate in December 1997 and left “fallow.” Not all of the lucerne was killed. Some re-growth of lucerne and grass occurred during the summer/autumn (December–May) of 1998 and it was necessary to spray the paddock again with glyphosate in the following April prior to sowing the canola.

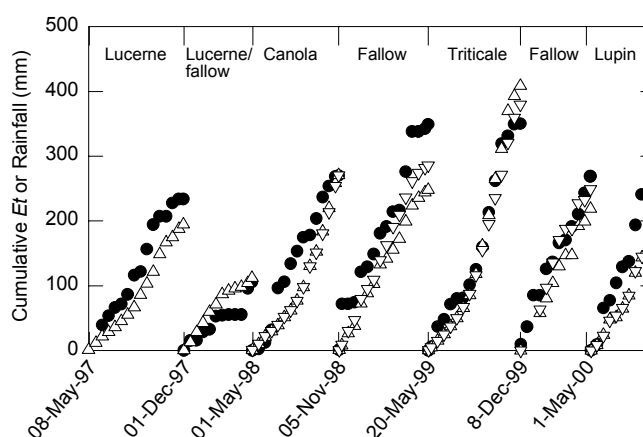


FIG. 3. Cumulative rainfall (●), Et estimated from the two lysimeters (north ▽ and south △) for the crop sequence from May 1997 to May 2000.

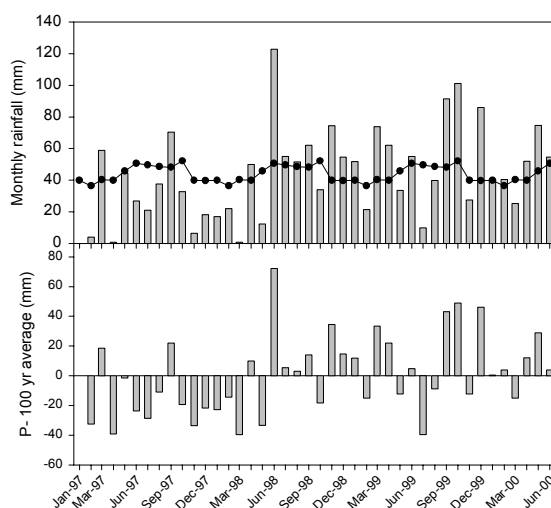


FIG. 4. Monthly rainfall ( $P$ ; vertical bars), average 100-year monthly rainfall (●) and the difference between monthly rainfall and the 100-year average monthly rainfall at the CSU site, Wagga Wagga from January 1997 to June 2000. (Data from NSW Agriculture.)

Cumulative Et from 1 December 1997 to 6 April 1998 was 98 mm, exceeding the rainfall of 55 mm. The summer/autumn re-growth not only used all the rainfall, but also 43 mm of stored moisture that had accumulated in the previous spring. Fifty mm of rain fell during April, and by 29 April 1998 cumulative Et (1 December 1997 – 29 April 1998) was 112 mm. Therefore, 36 mm of the depleted soil water storage was replenished.

The canola crop used all of the rain that fell from 30 April to 4 November 1998 (Fig. 3). Subsequent rainfall of 121 mm in November and December 1998 resulted in a significant increase in the water storage in the soil profile. Cumulative rainfall from 5 November 1998 to 20 May 1999 was 344 mm whereas Et was  $267 \pm 26$  mm (Fig. 2). In the following growing season, cumulative Et for the triticale crop exceeded the rainfall of 350 mm by  $44 \pm 19$  mm.

The climatic conditions during the experiments relative to long-term averages at the CSU site are shown in Fig. 4. Although rainfall at the other measurement sites was different from that at CSU, these data indicate the general relationship between rainfall during the measurement period and the long-term average. Monthly rainfall was below average for 10 months in 1997, near or above average for 7 months in 1998, above average for 7 months in 1999, and above average for 4 months to June 2000 (Fig. 4).

Variations in rainfall from year to year make it difficult to get an accurate picture of the effect of different cropping systems on water and chemical dynamics. Simulation modelling provides a means to explore these interactions, provided the models incorporate important processes adequately and can be shown to reproduce field behaviour within the bounds of experimental error. Simulations using APSIM (Agricultural Production Systems Simulator) [5–7] were compared with results from the field experiments at Charles Sturt University and found to agree closely. Exploratory simulations were then run on historical weather data (1957–1997) and confirmed the potential of lucerne to keep the soil drier and reduce drainage in a rotation. After removing the lucerne, soil water storage gradually increased under wheat, and was similar to that under continuous wheat after 2 to 3 years. The refilling of the buffer depends on the timing and amount of rainfall, as well as soil type and crop-rooting depth. For the scenario simulated, perhaps the wheat phase should not exceed 2 to 3 years. Additionally, the simulations show that drying out under lucerne occurs quickly and often within the first summer/autumn of the lucerne phase, with little difference in water use between lucerne and wheat during the winter/spring. These results suggest that practical benefits can be obtained by growing lucerne for as little as 12 months. In other years, when lucerne is slower to establish due to climatic conditions, it may need to be grown for 2 or even 3 years.

## REFERENCES

- [1] DUNIN, F.X., et al., Can agricultural management emulate natural ecosystems in recharge control across southern Australia? *Agrofor. Systems* **45** (1999) 343–364.
- [2] PECK, A.J., HURLE, D.H., Chloride balance of some farmed and forested catchments in southwestern Australia, *Water Resources Res.* **9** (1973) 648–657.
- [3] SMITH, C.J., Nitrate leaching from a Riverine clay soil under cereal rotation, *Aust. J. Agric. Res.* **49** (1998) 379–89.
- [4] KIRKEGAARD, J.A., et al., Comparison of canola, Indian mustard and Linola in two contrasting environments. II. Break-crop and nitrogen effects on subsequent wheat crops, *Field Crops Res.* **52** (1997) 179–191.
- [5] McCOWN, R.L., et al., APSIM: A novel software system for model development, model testing, and simulation in agricultural systems research, *Agric. Systems* **50** (1996) 255–271.
- [6] PROBERT, M.E., et al., APSIM's water and nitrogen modules and simulation of the dynamics of water and nitrogen in fallow systems, *Agric. Systems* **56** (1998) 1–28.
- [7] VERBURG, K., et al., SWIMv2.1 User Manual, Divisional Report 130, CSIRO Division of Soils, Australia (1996).

## FATE OF NITROGEN FROM MINERAL FERTILIZER AND LIQUID MANURE OVER A TWO-YEAR CROP ROTATION

P. CEPUDER

Institute of Hydraulics and Rural Water Management,  
University of Agricultural Sciences, Vienna, Austria

### Abstract

Nitrogen-15-labelled fertilizer was used to gain a better understanding of fertilizer-N uptake by crops in the year of application, the immobilization of applied N in the soil, fertilizer N uptake by a subsequent crop, and fertilizer-N leaching to groundwater. Fertilizer-N-utilization values for winter wheat (planted in the first year) were 15 to 36%, and 27 to 63% of the applied N was immobilized in the soil. Losses of fertilizer N by leaching to groundwater were not significant. At harvest of the winter wheat, between 50% and 82% of applied fertilizer N was accounted for in total. Unaccounted-for N was probably lost by volatilization. Only a few kilograms of applied N were taken up by the succeeding cover crop and corn.

### 1. INTRODUCTION

The intensification of agriculture within the past five decades has resulted in nitrate concentrations above the authorized limit in groundwater in many areas of Austria. The study presented here was carried out at Tullner Feld where nitrate levels in groundwater have been reported to be as high as 100 mg/L. To assess the contribution of various land uses to nitrate in groundwater, facilities for measuring nitrate leaching and percolation under various cropping and management systems have been installed by the Institute for Hydraulics and Rural Water-Management (BOKU, Vienna).

Due to agriculture-linked ecological deterioration of soil and water, N fertilization has, for some time, been a focal point of public discussion. Utilization and losses of fertilizer can be determined using isotope tracers. For agricultural and environmental studies, stable non-radioactive isotopes have particular utility. In nature, N isotopes of mass numbers 14 and 15 occur in the proportion 99.634 atom %  $^{14}\text{N}$  to 0.366 atom %  $^{15}\text{N}$ , therefore  $^{15}\text{N}$  enriched substances (especially fertilizers) are suitable for N-cycle and other studies [1].

The aims of this 2-year investigation were to employ  $^{15}\text{N}$  to gain a better understanding of fertilizer-N uptake by crops in the year of application, immobilization of the applied N in the soil, fertilizer N uptake by subsequent crops, and fertilizer-N leaching to groundwater.

### 2. MATERIALS and METHODS

#### 2.1. Field experiment – setup

The experiment was situated in the Tullner Feld Plain of Lower Austria. Groundwater level is approximately 5 m deep. Annual precipitation over 10 years has averaged 589 mm at the weather station at Tulln, and the mean temperature is 9.5°C, recorded at the Langenlebern station [2]. The predominant soil is a cernosem, a sandy loam/loam to a depth of 70 cm, from 70 to 90 cm a sandy-loamy silt, and from 90 to 115 cm a loam-sand/sandy silt. Below 115 cm, the main component is gravel. This soil has a plant-available storage capacity of approximately 170 mm of water. Humus content to 60 cm is 2 to 3%. Below 70 cm, the profile is humus deficient. The organic matter content varies between 0.4 and 0.6% (Table I).

This  $^{15}\text{N}$ -fertilizer experiment was inserted into a field trial that had been in progress since 1992. The long-term experiment consisted of six plots with dimensions of 10×5m (Fig. 1), each containing eight ceramic suction cups at depths of 45, 75, 105, and 135 cm in undisturbed soil, and one small lysimeter installed at 105 cm (Fig. 2).



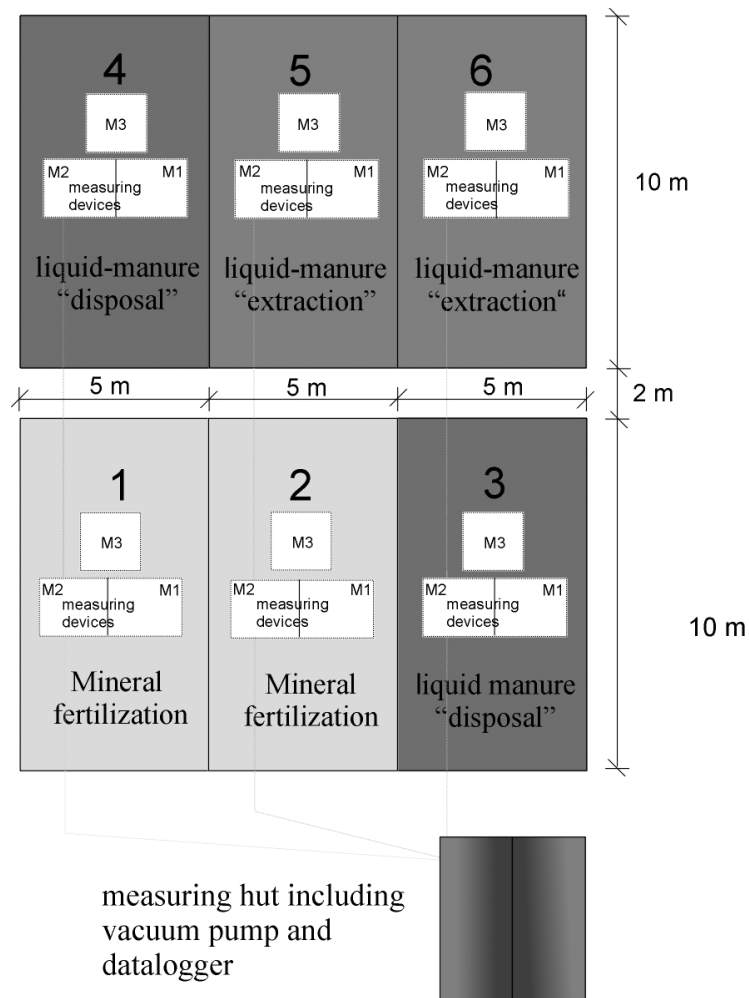


FIG. 1. The experimental set-up.

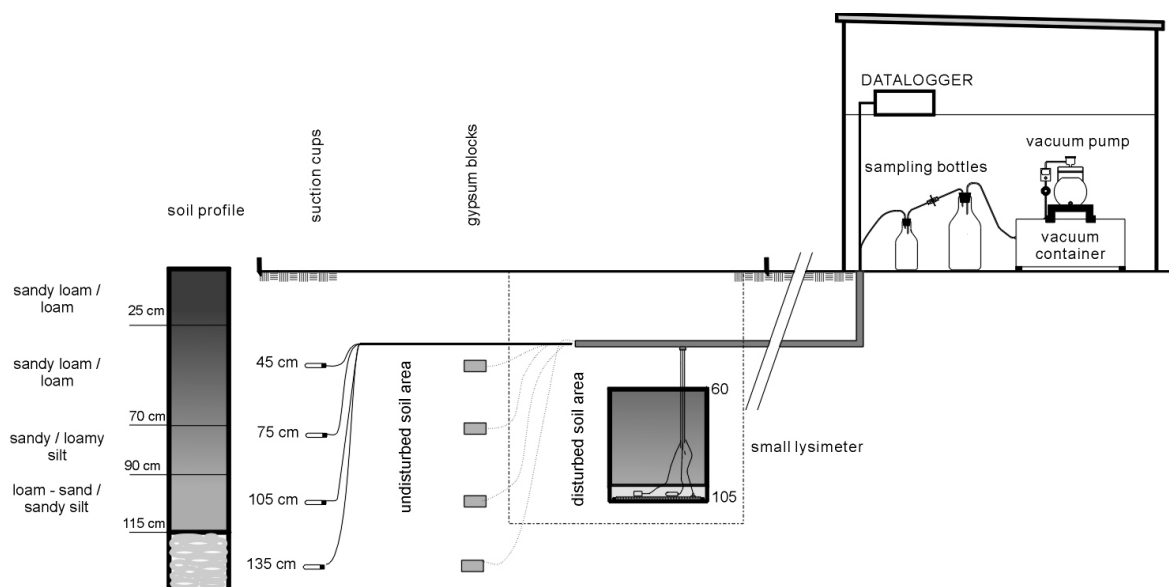


FIG. 2. Arrangement of the measuring devices

TABLE I. PHYSICAL AND CHEMICAL SOIL PARAMETER

Soil depth (cm)	Sand <sup>a</sup> (%)	Silt <sup>b</sup> (%)	Clay <sup>c</sup> (%)	Organic matter (%)	Available water (%)	kf-value <sup>b</sup> (m/d)
0–25	25	50	25	2.9	14	2.5
25–70	24	51	25	2.0	11	6.6
70–90	29	56	15	0.6	19	0.9
90–105	33	54	13	0.4	22	1.5

<sup>a</sup>2000–50  $\mu\text{m}$ . <sup>b</sup>50–2  $\mu\text{m}$ . <sup>c</sup><2  $\mu\text{m}$ . <sup>b</sup>Saturated hydraulic conductivity.

In the surface layer, above the measuring elements, 1-m<sup>2</sup> microplots were installed for the <sup>15</sup>N experiment. Microplots 1 (M1) and 2 (M2) were situated above suction cups and the small lysimeters (Fig. 1).

The study comprised three treatments. Paired plots were fertilized similarly (Fig. 1). Plots 1 and 2 were applied with the locally recommended rate of mineral fertilizer. Plots 3 and 4 were installed as “liquid-manure disposal” areas. These were fertilized twice a year (spring and autumn) consistent with the relatively low capacity of many liquid manure tanks in our rural regions. Extra mineral fertilizer could be applied, if conditions were sub-optimal for plant growth. Plots 5 and 6 were installed as “liquid manure extraction” areas. Liquid manure application to this area should, as for plots 1 and 2, fulfil crop needs for fertilizer. If N need was greater than that provided by the liquid manure, the shortfall was met with mineral fertilizer.

In the first year of the investigation (1997–98), winter wheat and a cover-crop mix (buckwheat, mustard and phacelia) were planted, and in 1999 corn was planted.

Nitrogen-15-labelled fertilizer was applied to the micro-plots when the winter wheat was fertilized. A 10% <sup>15</sup>N enriched ammonium sulphate was used instead of conventional ammonium sulphate [(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>] for plots 1 and 2. 0.5 g <sup>15</sup>N ammonium sulphate (98.2%) per liter was added to cattle liquid manure [3–5]; total N was thus increased by 3%. Hence the <sup>15</sup>N concentration of liquid manure was 2.5% in autumn 1997 and 2.6% in spring 1998. Table II gives an overview of the applied amounts and dates.

In total, the winter wheat was fertilized with 116 kg N/ha labelled fertilizer on plots 1 and 2, 286 kg N/ha on plots 3 and 4, and 204 kg N at plots 5 and 6.

After the harvest of wheat in July 1998, the cover crop was planted to exploit any plant-available N, and incorporated into the soil in November 1998, so that the assimilated N remained in the field for possible uptake by the subsequent corn.

No labelled fertilizer was used with autumn liquid-manure applications in 1998 to plots 3 and 4, and mineral fertilization of corn in the spring of 1999 was as shown in Table II.

## 2.2. Analyses

To determine uptake of fertilizer N by plants, roots, shoot, and grains were analysed. Plant samples were dried to constant weight (70°C) and milled. Total-N content of each sub-sample was determined by Kjeldahl digestion [6] then <sup>15</sup>N enrichment determined on the digestate.

After harvest, soil samples were taken from the microplots on the M3 areas (Fig. 1) to determine immobilized N. A combined soil sample for the 0 to 30 cm layer was excavated. Samples for depths of 30 to 60, 60 to 90, and 90 to 120 cm were obtained by drilling. To determine total N by Kjeldahl digestion, soil samples were dried (105°C) to constant weight and ground. Subsamples of digestate were taken for <sup>15</sup>N analysis.

TABLE II. LABELLED AND UNLABELLED FERTILIZER APPLICATIONS TO WINTER WHEAT AND CORN

Timing	Plot and treatment		
	1+2 Mineral fertilizer	3+4 Liquid manure “disposal”	5+6 Liquid manure “extraction”
24.10.1997 (labelled)	—	30 m <sup>3</sup> /ha 130 kg N/ha (44 kg NH <sub>4</sub> -N)	30 m <sup>3</sup> /ha 130 kg N/ha (44 kg NH <sub>4</sub> -N)
26.3.1998 (labelled)	42 kg N/ha	32 kg N/ha <sup>a</sup>	32 kg N/ha <sup>a</sup>
20.4.1998 (labelled)	—	30 m <sup>3</sup> /ha 124 kg N/ha (73 kg NH <sub>4</sub> -N)	—
7.5.1998 (labelled)	42 kg N/ha	—	42 kg N/ha <sup>a</sup>
2.6.1998 (labelled)	32 kg N/ha	—	—
16.11.1998 (not labelled)	—	30 m <sup>3</sup> /ha 114 kg N/ha (44 kg NH <sub>4</sub> -N)	—
28.4.1999 (not labelled)	—	15 m <sup>3</sup> /ha 63 kg N/ha (36 kg NH <sub>4</sub> -N)	15 m <sup>3</sup> /ha 63 kg N/ha (36 kg NH <sub>4</sub> -N)
28.4.1999 (not labelled)	120 kg N/ha	54 kg N/ha <sup>a</sup>	40 kg N/ha <sup>a</sup>

<sup>a</sup>Extra mineral fertilizer application to liquid manure plots.

Downwards transport of fertilizer N was observed with the help of the suction cups at depths of 105 and 135 cm, and with the small lysimeters. Samples were taken weekly. First, N was determined and then soil water was prepared for <sup>15</sup>N analysis. The modified diffusion method for soil solutions containing <sup>15</sup>N-labelled mineral N was used [7]. Nitrate (NO<sub>3</sub><sup>-</sup>) in the soil solution was converted to ammonium (NH<sub>4</sub><sup>+</sup>) using Devarda’s alloy. Alkaline medium (1.0 N NaOH) caused NH<sub>3</sub>-gas to be released from the solution. The NH<sub>3</sub>-gas was collected as NH<sub>4</sub><sup>+</sup> on an acidified (2.5 N KHSO<sub>4</sub>) filter disc (Whatman GF/D, 10 mm diameter). After a minimum 16 h of diffusion, the filter discs were transferred to tin capsules for analysis in a Carlo Erba automated Dumas combustion system connected to a VG-SIRA mass spectrometer, at the IAEA laboratories, Seibersdorf, Austria.

The fraction of N derived from fertilizer (Q<sub>NF</sub>) was calculated according to the following equation (1):

$$Q_{NF} = \frac{Q_N \cdot E}{E_0} \quad (1)$$

where

Q<sub>N</sub> total nitrogen content (%),  
E measured <sup>15</sup>N-enrichment in the sample (%),  
and E<sub>0</sub> <sup>15</sup>N-enrichment of the fertilizer (%).

Nitrogen losses due to ammonia volatilization [7] were captured by a modified semi-open version of the device described in Ref. [9], which consisted of a plastic tube with an inside diameter of 145 mm. Two metal bars were built into the tube at different positions on which pads of foam (diameter 145 mm, thickness 26 mm) rested. Over this device a cover (35 cm above soil) was attached for rain protection.

TABLE III. FERTILIZER APPLIED, YIELD, TOTAL NITROGEN AND FERTILIZER NITROGEN UPTAKE BY WINTER WHEAT

Plot	Fert. applied (kg N/ha)	Grain yield (t/ha)	Total uptake (kg N/ha)	Fert. uptake (kg N/ha)	Fert. utilization (%)
1	116	4.1 ± 0.2	137	37	32
2	116	5.0 ± 0.5	178	42	36
3	286	5.0 ± 0.7	165	46	16
4	286	4.6 ± 0.5	156	44	15
5	204	4.0 ± 0.5	117	49	24
6	204	5.0 ± 0.8	150	59	29

Foam pads were washed with distilled water three times before installation, then soaked with a solution of phosphoric acid and glycerin and transported to the measuring site in airtight containers.

After a predetermined time interval, these ammonia-absorbing pads were exchanged quickly for new ones and taken to the laboratory in airtight containers and rinsed with distilled water three times. To calculate the collected quantity of  $\text{NH}_4\text{-N}$ , a sub-sample of the solution was distilled with sodium hydroxide, collected in boric acid and back-titrated with hydrochloric acid. Investigations were executed up to no measurable quantities of  $\text{NH}_4\text{-N}$  were absorbed by the pads.

### 3. RESULTS

Table III shows grain yields, total N and fertilizer-N uptake and utilization by winter wheat. Grain yields varied between 4.0 and 5.0 t/ha. Nitrogen accumulation by the grain amounted to between 91 and 127 kg/ha. Total-plant N accumulation was 117 to 178 kg/ha.

Fertilizer uptake was between 37 and 59 kg N/ha (Table III); plot 1 provided the smallest quantity and plot 6 the largest. Most of the N assimilated by the winter wheat came from the soil (i.e. 68 to 136 kg N/ha); fertilizer N contributed 27% and 24%, in plots 1 and 2, 28% in plots 3 and 4, and 42% and 39% in plots 5 and 6, respectively.

Fertilizer utilization, i.e. the amount of fertilizer N taken up as a fraction of the amount applied, varied between 15% and 36% (Table III). Actual fertilizer utilization was between 11% and 29% because straw and roots remained on the field. Mineral fertilizer N was more efficiently utilized than liquid manure N by winter wheat—utilization values for plots 5 and 6 (fertilized twice with ammonium sulphate and once with liquid manure in the autumn) were twice as high as those for plots 3 and 4 (one mineral fertilizer application and two of liquid-manure in autumn and spring).

Table IV shows amounts and fractions of fertilizer N that were recovered from the soil after winter wheat. Between 31 and 142 kg/ha of fertilizer N were recovered from the soil, i.e. between 27 and 50% of the applied N remained in the soil after the winter wheat was harvested. And the amounts of fertilizer N in the soil remained largely unchanged until the end of the experiment.

At the winter-wheat harvest (July 1998), of the  $^{15}\text{N}$  applied, between 50% and 82% was recovered as plants and soil, between 73 up to 186 kg /ha of fertilizer N. At that time no N losses due to leaching occurred.

From plots 1 and 2, 95 kg N/ha and 73 kg N/ha were recovered (in the winter wheat crop and soil), respectively, of the applied 116 kg N/ha fertilizer applied. Nitrogen losses due to volatilization ranged between 7 to 8 kg/ha at the first spring application of fertilizer. Comparable volatilization losses for the further application of fertilizer would explain the N-budget shortfall.

TABLE IV. FERTILIZER N APPLIED AND RECOVERED FROM SOIL

Plot	Fertilizer rate (kg N/ha)	Recovered fertilizer (kg N/ha)	Recovery rate (%)
1	116	58	50
2	116	31	27
3	286	98	34
4	286	142	50
5	204	70	34
6	204	67	33

On plots 3 and 4, 50 and 65%, respectively, of the 286 kg N/ha applied (liquid manure “disposal”) were recovered in the crop and soil. Nitrogen losses due to volatilization were 15% in the autumn, and 25% and 10% in March and April 1998, respectively. These results imply losses of 8 to 13% of the total applied N and may be explained if available ammonium sulphate N from manure applied in the autumn of 1997 (44 kg  $\text{NH}_4\text{-N/ha}$ ) and in the spring of 1998 (73 kg  $\text{NH}_4\text{-N/ha}$ ) was volatilized.

Recovery rates of 58 to 62% were observed for the liquid-manure “extraction” plots 5 and 6. Volatilization losses were 15% in the autumn of 1997, and 25% in the spring of 1998. Further measurements were not made. Therefore 12% loss of total applied N was calculated. Using the same considerations as for plots 3 and 4, different quantities of total applied N can be explained by the volatilization of 44 kg N/ha as ammonium sulphate.

Amounts of fertilizer-N uptake by winter wheat, the cover crop and corn over the 2-year period and those immobilized in the soil are presented in Table V.

The initial application of fertilizer resulted in immobilization of 31 to 142 kg/ha during the growth of the winter wheat. Straw and roots remained in the soil. As the uptake of the N applied to winter wheat by the cover crop was relatively small, approximately the same amounts of N were measured in soil after ploughing in the cover crop. After harvest of the corn, similar amounts of fertilizer N were found in soil. Because there was little remobilization of the immobilized fertilizer N during the following cropping periods, the movement of this N into deeper soil layers was commensurately slight. Seventy to 90% remained in the surface soil layer and 3 to 19% were measured in the 30- to 60-cm layer.

TABLE V. FERTILIZER N (kg/ha) IMMOBILIZED AND TAKEN BY CROPS OVER 2 YEARS

Plot	Winter wheat				Cover crop			Corn			
	Grain	Straw + root	Soil	Total	Plant	Soil	Total	Grain	Straw + root	Soil	Total
1	29	8	58	66	5	57	62	2	1	49	52
2	34	8	31	39	6	41	47	3	1	38	42
3	32	14	98	112	9	89	98	4	5	80	89
4	33	11	142	153	11	115	126	5	4	122	131
5	39	10	70	80	3	70	73	3	3	59	64
6	47	12	67	79	6	68	74	3	3	65	71

The amount and distribution of rainfall are important parameters for percolation and leaching. In Fig. 3 the weekly and total amounts of rainfall during the investigation period are shown (the end of October 1997 to the end of September 1999). The year 1997 was rather wet with a total of 653 mm. During the last 2 months of 1997, after the liquid-manure application to plots 3 to 6, rainfall was 104 mm. Total precipitation was 546 mm in 1998, and before the end of the investigation another 552 mm were recorded.

In order to determine the proportion of the fertilizer N applied to winter wheat as total discharge, water samples from suction cups at 105 cm were analysed for nitrate and  $^{15}\text{N}$  concentration, and small lysimeters were used to calculate total-N and fertilizer-N leaching. The measurements were made weekly during the investigation period and when percolation occurred. As no percolation was registered after July 1998 the sampling was stopped. From the middle to the end of 1997, also, no percolation occurred.

For each of the three fertilizer uses in two replications, the nitrate and  $^{15}\text{N}$  concentrations at 105 cm are shown in Figs. 4 to 6. The figures also show cropping periods, dates of fertilization, total percolation, and total and fertilizer N in leachate. Percolation varied between 131 and 211 mm, which means that values between 11 and 18%, inclusive, of total rainfall (1,202 mm) were measured for the 2-year period.

As plot 1 received no fertilizer from 1992 to 1996, the nitrate concentration in the percolated solution was relatively high during the first year. In 1999 the concentration decreased due to increased percolation. And as the split mineral-fertilizer applications were made in the spring of 1998, no leaching of fertilizer N occurred until the start of next leaching period at the end of 1998. The measured concentration of  $^{15}\text{N}$  increased to 0.35% though the concentration of nitrate in percolation decreased. Only up to 0.3 kg /ha of leached fertilizer N was recorded, although total N leaching was between 3 and 9 kg/ha.

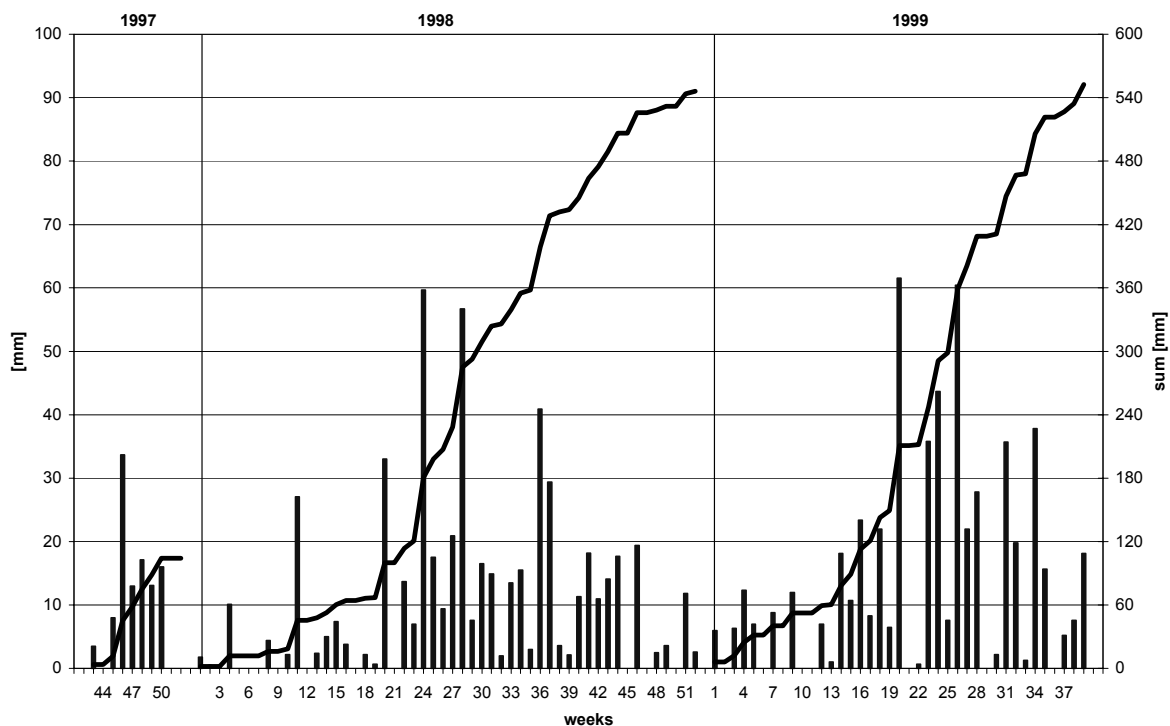


FIG. 3. Weekly amounts and yearly sums of rainfall

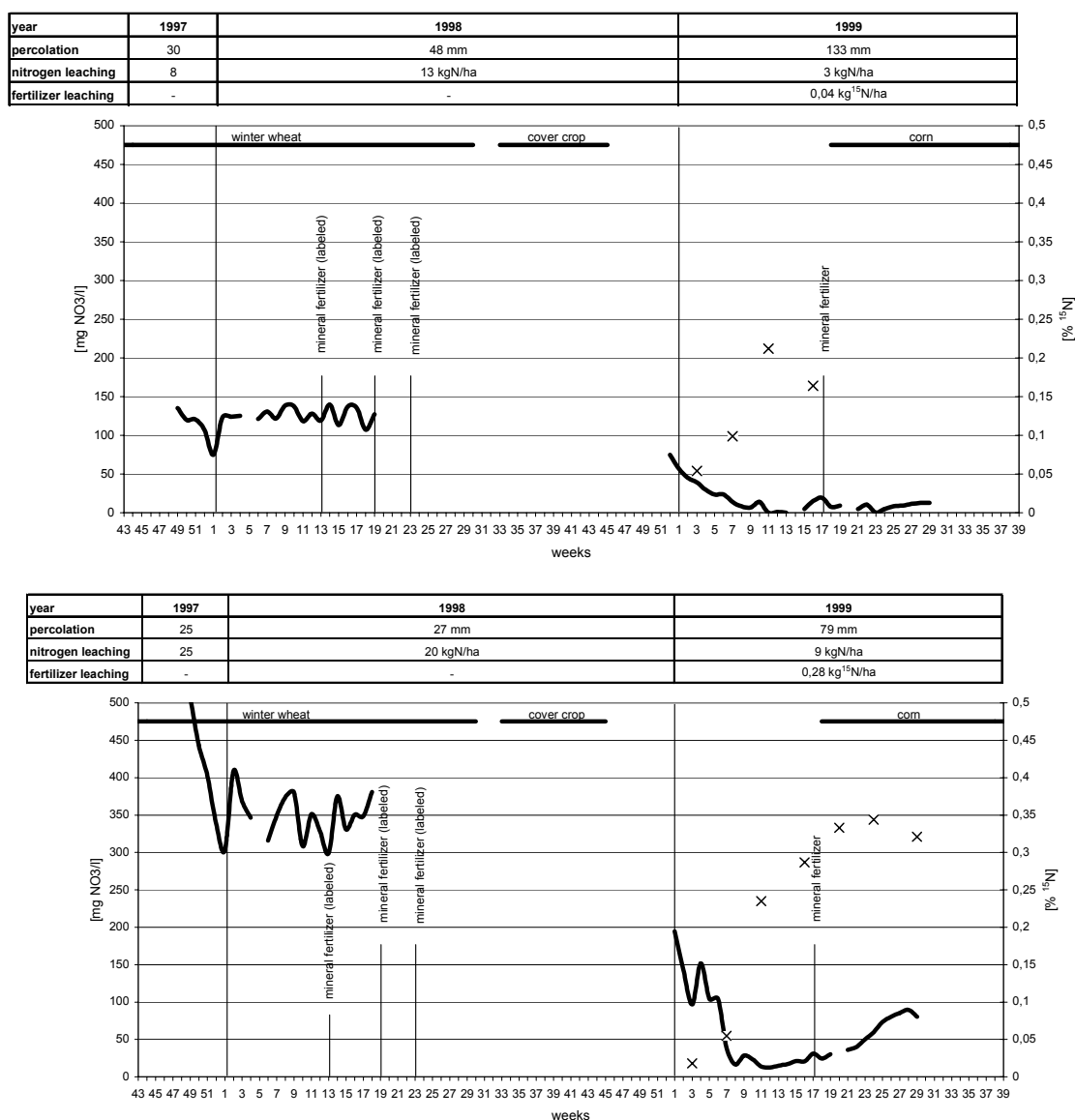
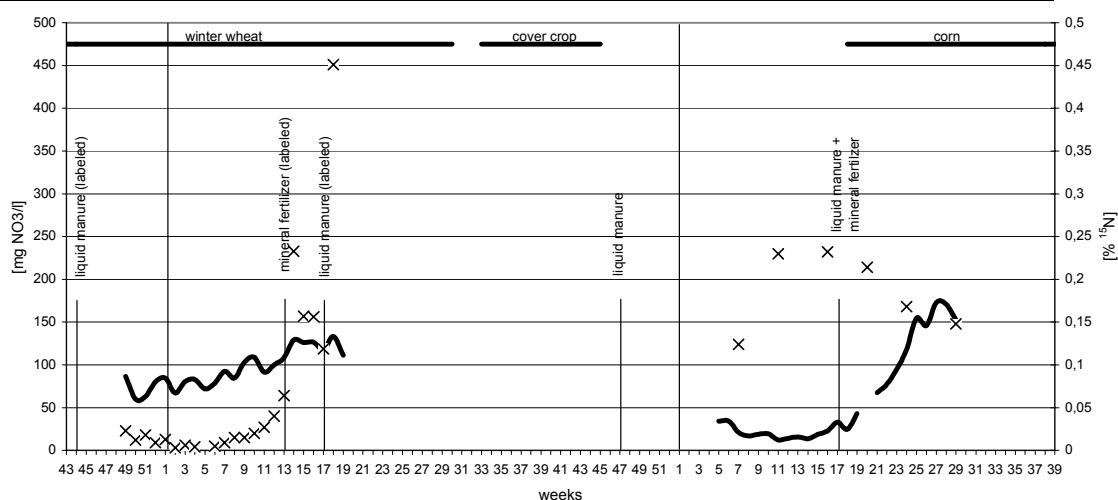


FIG. 4. Nitrate concentration, <sup>15</sup>N concentration in percolation, percolation, N leaching and fertilizer-N leaching for plots 1 (above) and 2 over the 2-year period. (Line = mg NO<sub>3</sub>/L, x = <sup>15</sup>N‰.)

In the period 1992 to 1996, fertilizer application to plot 3 was reduced (50% of the recommended value) and increased for plot 4 (130%). Therefore, in 1998, the nitrate concentration in percolation water was higher for plot 4. In 1999, the nitrate concentration was very low and increased to 150 mg/L before the end of the percolation period. As labelled liquid-manure applications were initiated in the autumn of 1997, <sup>15</sup>N measurements were possible in the spring of 1998. But the <sup>15</sup>N concentrations were very low and leached fertilizer was only about 0.1 kg N/ha. In 1999 the <sup>15</sup>N concentrations in percolation increased to 0.25%, but due to low nitrate concentration the leached N from the liquid manure on the “disposal plot” was also only 0.5 kg/ha. Leached N as a whole reached 9 to 11 kg/ha.

From 1992 to 1996, plot 5 was unfertilized with no crops (bare soil), during which N was lost by leaching and the soil became low in nitrate. On plot 6, liquid-manure “extraction” had been practised since 1992. The average nitrate concentrations from week 49 of 1997 to week 19 of 1998 were 84 and 116 mg/L in plots 5 and 6, respectively; no leached fertilizer N was registered. In 1999, the average nitrate concentrations were 5 and 43 mg/L, respectively. Due to the low nitrate concentration in plot 5, no leached liquid manure-N was detected. In plot 6 the leached N was 11 kg/ha, and 0.4 kg/ha of liquid manure N was measured.

year	1997	1998	1999
percolation	39	28 mm	87 mm
nitrogen leaching	6	6 kgN/ha	11 kgN/ha
fertilizer leaching	0,04	0,13 kg <sup>15</sup> N/ha	0,55 kg <sup>15</sup> N/ha



year	1997	1998	1999
percolation	34	24 mm	75 mm
nitrogen leaching	36	17 kgN/ha	9 kgN/ha
fertilizer leaching	0,05	0,04 kg <sup>15</sup> N/ha	0,54 kg <sup>15</sup> N/ha

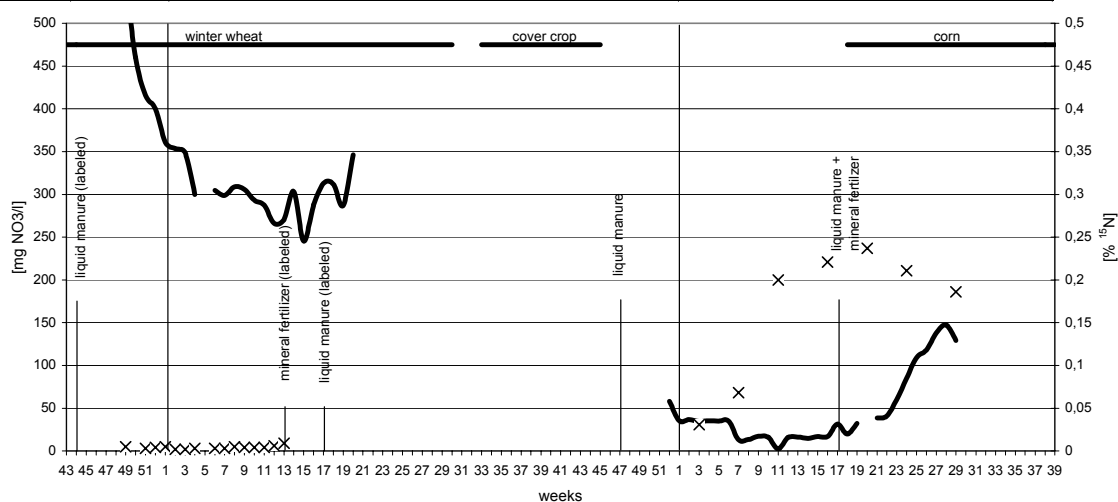


FIG. 5. Nitrate concentration, <sup>15</sup>N concentration in percolation, percolation, N leaching and liquid-manure-N leaching for plots 3 (above) and 4 over the 2-year period. (Line = mg NO<sub>3</sub>/L, x = <sup>15</sup>N%).

#### 4. SUMMARY

Fertilizer-N-utilization values for winter wheat (planted in the first year) were 15 to 36%. At harvest 27 to 63% of the applied N was immobilized in the soil. Losses of fertilizer N by leaching to groundwater were not significant until harvest of the winter wheat.

At harvest of the winter wheat, between 50% and 82% of applied fertilizer N was recovered in total. Unaccounted-for N was probably lost by volatilization.



Only a few kilograms of N were taken up by the succeeding cover crop and corn. Comparable quantities of immobilized fertilizer N, as determined at harvest of the winter were recorded after the corn crop, most of it in the 0- to 30-cm layer.

The leaching of applied mineral N and liquid manure N amounted to a maximum of 1 kg/ha during the entire investigation period.

## ACKNOWLEDGEMENTS

The Institute of Hydraulics and Rural Water Management thanks the Austrian Academy of Science for financing the project and the laboratories of the FAO/IAEA joint Division at Seibersdorf for the  $^{15}\text{N}$  measurements.

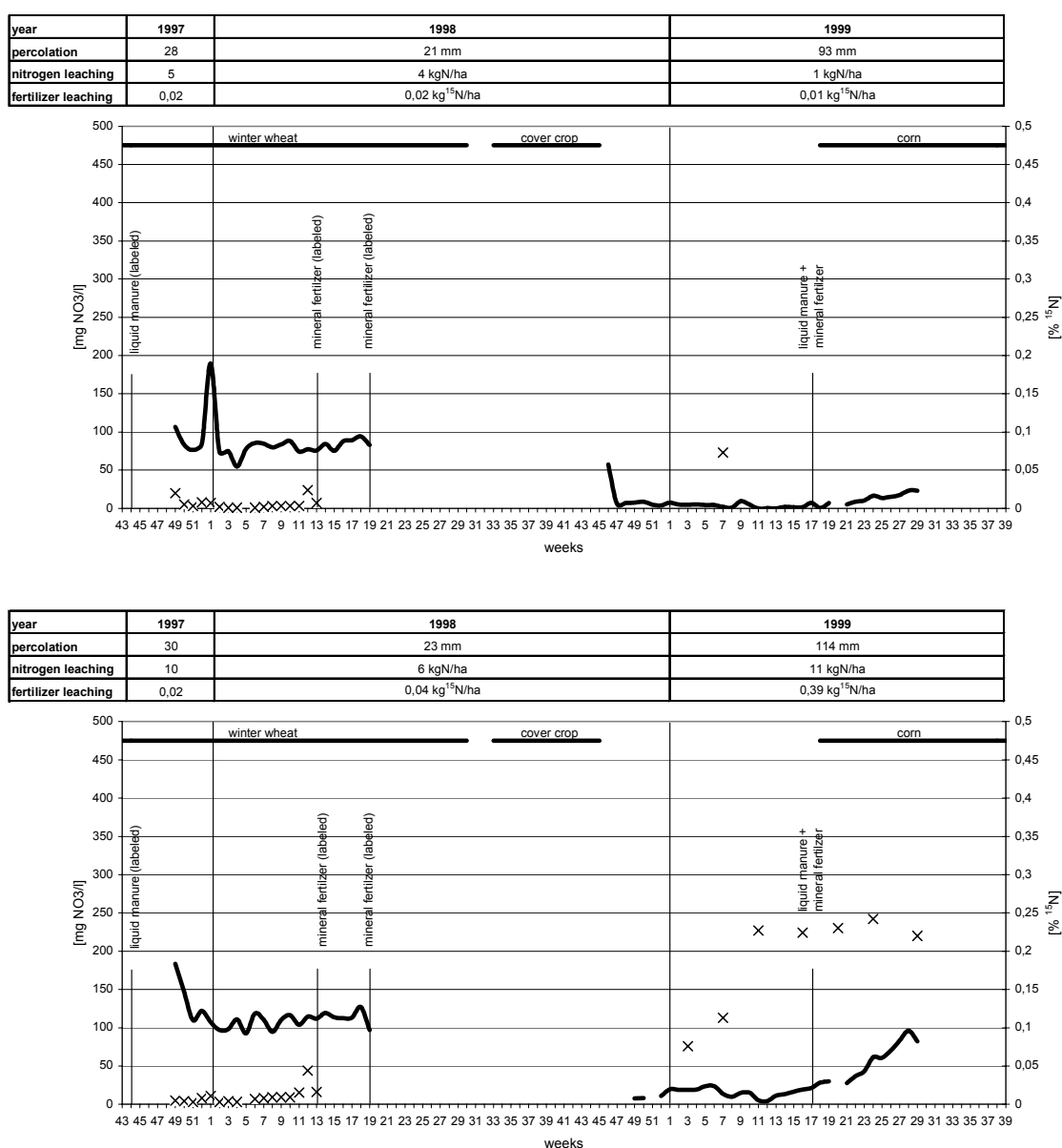


FIG. 6. Nitrate concentration,  $^{15}\text{N}$  concentration in percolation, percolation, N leaching and liquid-manure-N leaching for plots 5 (above) and 6 over the 2-year period. (Line = mg  $\text{NO}_3/\text{L}$ ,  $\times$  =  $^{15}\text{N}\%$ )

## REFERENCES

- [1] INTERNATIONAL ATOMIC ENERGY AGENCY, Use of Nuclear Techniques in Studies of Soil-Plant Relationships, TCS 2, IAEA, Vienna (1990).
- [2] HYDROGRAPHISCHER DIENST IN ÖSTERREICH, Die Niederschläge, Schneeeverhältnisse und Lufttemperaturen in Österreich im Zeitraum 1981–1990. Beiträge zur Hydrographie Österreichs, Heft Nr. 52, Hydrographisches Zentralbüro im BMLF, Vienna (1994).
- [3] RECOUS, S., et al., The fate of labeled  $^{15}\text{N}$  urea and ammonium nitrate applied to a winter wheat crop, *Plant Soil* **112** 205–214 (1988).
- [4] PAUL, J.W., BEAUCHAMP, E.G., Availability of manure slurry ammonium for corn using  $^{15}\text{N}$ -labelled  $(\text{NH}_4)_2\text{SO}_4$ , *Can. J. Soil Sci.* **75** (1994) 35–42.
- [5] LIPPOLD, H., NEBE, D., Verlagerung von  $^{15}\text{N}$ -markiertem Nitrat im Winterhalbjahr und nachfolgende Verwertung durch Winterweizen im Feldexperiment auf verschiedenen Standorten, *Agribiol. Res.* **47** (1994) 3–4.
- [6] ÖNORM, L., Bestimmung von Gesamtstickstoff, Österreichisches Normungsinstitut, Vienna (1988).
- [7] BROOKS, P.D., et al., Diffusion Method to Prepare Soil Extracts for Automated Nitrogen-15 Analysis, *Soil Sci. Soc. Am. J.* **53** (1989) 1707–1711.
- [8] THOMPSON, R.B., Denitrification in slurry-treated soil: occurrence at low temperatures, relationship with soil nitrate and reduction by nitrification inhibitors, *Soil Biol Biochem* **21** (1989) 875–882
- [9] MARSHALL, V.G., DEBELL, D.S., Comparison of four methods of measuring volatilization losses of nitrogen following urea fertilization of forest soils, *Can. J. Soil Sci.* **60** (1980) 549–563.

# **EFFECT OF NUTRIENT AND SOIL MANAGEMENT ON THE EFFICIENCY OF NITROGEN AND WATER USE IN RAINFED WHEAT IN CHINA**

GUIXIN CAI

Institute of Soil Science,  
Chinese Academy of Sciences,  
Nanjing

TINGHUI DANG, SHENGLI GUO, MINGDE HAO

Institute of Soil and Water Conservation,  
Chinese Academy of Sciences,  
Yangling

China

## **Abstract**

Two field experiments on rainfed wheat were conducted in the southern part of the Chinese Loess Plateau, from September 1998 to June 1999. One experiment compared four N-fertilizer treatments: CK (without N), N1 (100 kg N/ha as urea), N1O (100 kg N/ha as urea and 50 kg N/ha as organic manure) and N2 (150 kg N/ha as urea). An  $^{15}\text{N}$  study was carried out within these three treatments. The second experiment compared four mulching treatments. Grain yield was significantly increased by application of N fertilizer. The yield was 2.77 t/ha in the CK treatment, and it was increased to 3.99, 4.3, and 3.73 t/ha with N1, N1O, and N2, respectively. Yields were not significantly affected by increasing the application rate from 100 to 150 kg N/ha with either additional urea or manure. The  $^{15}\text{N}$  study showed that plant recoveries were approximately 38% of the applied N; N remaining in the 0- to 40-cm soil layer ranged from 29 to 34% and the proportion of N unaccounted for also was 29 to 34%. Recoveries were not affected by application rate of N. Water use efficiency (WUE) for CK was the lowest (7.6 kg/ha/mm), and it increased to 9.7 to 12 kg/ha/mm for the fertilized treatments. The wheat extracted more water from the 1- to 2-m depth (47–60% of the total soil water depletion from 0–3 m) than from 0 to 1 m (33–40% of the total soil water depletion from 0–3 m). Plastic-film and straw mulching had no significant effects on wheat yield.

## **1. INTRODUCTION**

This study is a part of an IAEA Co-ordinated Research Project (CRP), “Management of Nutrients and Water in Rainfed Arid and Semi-Arid Areas for Increasing Crop Production.” In keeping with the objectives of the CRP, field experiments involving isotope techniques were designed and set up to study the effects of fertilizer-N management and mulching on water use efficiency and responses to N in rain-fed wheat. This paper summarizes the results obtained in the first year of the experiment in northern China.

## **2. EXPERIMENTS AND TREATMENTS**

Field experiments involving isotopes were conducted at Changwu Experimental Station, located in the southern part of the Chinese Loess Plateau. The experiments were initiated in September 1998, and the project will continue until 2002. The study includes two experiments, each with four treatments in four replicates with a Latin-square design. The area of each plot is 42 m<sup>2</sup>. Phosphorus at a rate of 35 kg/ha of was applied to all treatments. All of fertilizers, including N, P, and organic fertilizer, were applied in the first wheat-cropping season. Before sowing, fertilizers were broadcast on the soil surface followed by ploughing, resulting in an incorporation depth of 15 to 20 cm for most of the fertilizers. After harrowing, wheat seeds were sown with a 20-cm row spacing at about 5 cm depth at a rate of 180 kg/ha.

## 2.1. Experimental site

Changwu Experimental Station is located in Changwu County, Shaanxi Province, China (35°12'N, 107°40'E, elevation 1,200 m), in the southern part of the Chinese Loess Plateau. The mean annual rainfall is 580 mm, mean annual temperature is 9.1°C, annual cumulative mean daily temperature >10°C is 3,030°C, annual duration without frost is 170 days, and annual sunshine time is 2,230 h.

The soil, named Heilu, is derived from loess with a deep and even profile. The surface layer (0–20 cm) has a pH of 8.4, CaCO<sub>3</sub> content of 10%, total N content of 0.8 g/kg, available P 14 mg/kg, available K 146 mg/kg, cation exchange capacity of 13 cmol/kg, bulk density of 1.3 g/cm<sup>3</sup>, field capacity of 27%, and <0.01 mm clay 37%.

## 2.2. Treatments

The first experiment was composed of the following treatments.

- CK: no applied N,
- N1: 100 kg N/ha as urea,
- N2: 150 kg N/ha as urea,
- N1O: 100 kg N/ha as urea and 50 kg N/ha as organic manure.

The second experiment was composed of the following four mulch treatments.

- W: traditional planting, i.e. plane planting with a row spacing of 20 cm,
- WM1: mulching and ridge planting 1. The ridge was 30-cm wide and covered with white plastic film without planting, and four rows of wheat seeds were sown between two ridges with spacing of 15 cm,
- WM2: mulching and ridge planting 2. The ridge was 60-cm wide and covered with plastic film. Wheat seeds were sown under the film in four rows (15-cm spacing), the distance between two ridges was 30 cm without planting,
- WT: no-tillage after harvesting of the wheat and the soil mulched with straw during the fallow season (late June to late September).

Urea was applied at 150 kg N/ha to all the mulch treatments and W.

## 2.3. <sup>15</sup>N microplot experiment

Experiment 1 included an <sup>15</sup>N-labelled urea component in the three treatments of N1, N2 and N1O with four replicates. The microplots were bounded by iron frames with dimensions of 50×40×60 cm (height). The frames were inserted into the soil leaving 5 cm above the surface. The abundance of <sup>15</sup>N in the urea was 7.06 atom %. Soil and plant samples were taken at physiological maturity and analysed for total N and <sup>15</sup>N at the IAEA laboratory in Seibersdorf, Austria.

## 2.4. Measurement of soil water content

At sowing and harvesting, soil samples in the 0- to 300-cm profile were oven-dried at 105°C to a constant weight, to determine gravimetric water content. This was converted it to volumetric water content according to soil bulk density. During the cropping season, the soil water content was periodically measured using a neutron moisture meter calibrated against the actual determinations of volumetric water contents.

### 3. RESULTS AND DISCUSSION

#### 3.1. Wheat response to nutrient application

The CK treatment yielded 2.77 t/ha of wheat grain. Yields were 3.99, 3.73, and 4.30 t/ha (increases of 44, 35, and 55%) with treatments N1, N2, and N1O, respectively (Table I). There were no significant differences among the yields of the fertilized treatments, indicating that 100 kg N/ha was adequate for the rain-fed wheat with limited water supply in that year [1].

The Productivity Indices (PI, i.e. kg/ha of additional yield above CK per kg of plant nutrient applied) with treatments N1 and N1O were higher than with N2 (Table I). Also, the harvest indices (grain yield over total dry matter yield) in treatments N1 and N1O were also higher than that in N2. The relatively high PI values (10 to 12 kg/kg N) with 100 kg N/ha as urea with or without organic manure at 50 kg N/ha show that the application of 150 kg N/ha as urea (PI = 6.4) was too high for rain fed wheat in that year. The PIs for N obtained in this experiment were similar to others obtained at the Chinese Agricultural Academy of Science for wheat [2], where PI decreases with increasing N-application rates have also been obtained.

#### 3.2. Effect of mulching on crop yield

There was no effect of mulching treatment on grain yield in the first cropping season (Table II). Mulching is a management practice for improving water infiltration and reducing evaporation. However, in this experiment, neither of the treatments using plastic increased grain yield, and there was a trend for straw weight to decrease. One possible reason was that the plant density with plastic mulch was 10 to 15% lower than that with traditional planting (see 2.2). Nevertheless, the effect of mulch on crop yield should be tested over a range of seasonal conditions.

TABLE I. WHEAT RESPONSE TO UREA APPLICATION

Treatment	Grain yield (t/ha)	Increase (%)	Productivity index (kg/kg N)	Straw wt (t/ha)	Increase (%)	Harvest index
CK	2.77b <sup>a</sup>	—	—	5.64b	—	0.33
N1	3.99a	44	12.1	6.17ab	9.4	0.39
N2	3.73a	35	6.40	7.33a	30	0.34
N1O	4.30a	55	10.2	6.24ab	11	0.41

<sup>a</sup>Means within a column followed by the same letter are not significantly different ( $P=0.05$ ) by Duncan's multiple-range test.

TABLE II. EFFECT OF MULCHING ON WHEAT YIELD

Treatment	Grain yield (t/ha)	Increase (%)	Straw weight (t/ha)	Increase (%)	Harvest index
W	4.21a		7.87a		0.35
WM1	4.56a	8.3	6.44b	-18	0.41
WM2	4.00a	-5.0	6.52ab	-17	0.38
WT	4.25a	1.0	7.37ab	-6.4	0.37

<sup>a</sup>Means within a column followed by the same letter are not significantly different ( $P=0.05$ ) by Duncan's multiple-range test.

### 3.3. Dynamics of volumetric soil water content and efficiency of water use

Crop yield is greatly affected by annual rainfall in rainfed regions. For example, the average wheat yield at Changwu county was 2.38 t/ha in 1994 with a normal annual rainfall (576 mm) and 2.86 t/ha in 1991 with a higher rainfall (666 mm), but only 0.65 t/ha in 1995 with a lower rainfall (318 mm).

Soil water content at harvesting was much lower than at sowing (Fig. 1). At sowing, the mean volumetric water content of the 0- to 100-cm soil layer was  $0.251 \text{ cm}^3/\text{cm}^3$ , and it decreased to 0.189 to  $0.209 \text{ cm}^3/\text{cm}^3$  for different treatments at harvesting. The corresponding figure for the 100–200 cm layer was  $0.257 \text{ cm}^3/\text{cm}^3$  at sowing and 0.181 to  $0.187 \text{ cm}^3/\text{cm}^3$  at harvesting. The water contents with the different treatments showed little variation through the growing season.

Net depletion of soil water during the cropping season can be calculated from the change in soil water content, and the results for the various treatments are listed in Table III. Rainfall during wheat growth was 242 mm. Total consumption of water was calculated from the sum of soil-water depletion throughout the 3-m profile and rainfall, and water use efficiency (WUE) was calculated using total water consumption. Table III shows that WUE was lowest (7.6 kg/ha/mm) for CK. WUE increased to 9.70 to 11.7 kg/ha/mm for the treatments with application of N fertilizer. The highest WUE was obtained with the WM1 treatment (mulching and ridge planting 1). Previous studies in the region showed that WUE is low (2.6–6.5 kg/ha/mm) without N fertilization and may reach 13 kg/ha/mm with fertilization [3]. Results from Zhou [4] showed that the average WUE for rain-fed wheat in the Fengqiu region was 7.2 kg/ha/mm, and it increased to 11 to 17 kg/ha/mm with fertilization. In the present experiment, soil water from the 1- to 2-m depth interval (47–60% of total soil water depletion in 0–3 m) contributed more to wheat crop water use than soil water in the 0- to 1-m interval (33–40% of total soil water in 0–3 m).

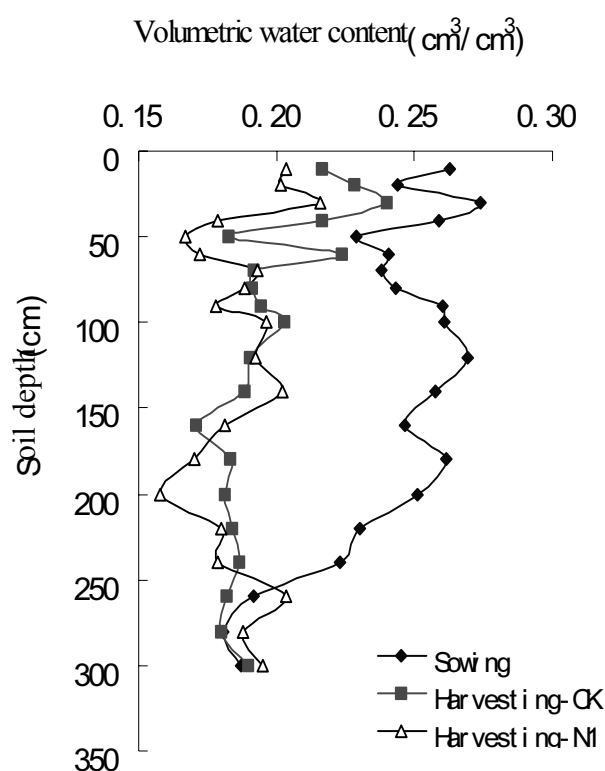


FIG. 1. Volumetric water content in the soil profile (0–300 cm) at sowing and harvesting.

TABLE III. EFFECTS OF NITROGEN AND MULCH TREATMENTS ON WATER CONSUPTION, GRAIN YIELD AND WATER USE EFFICIENCY

Component/Soil layer		CK	N1	N1O	N2	WM1	WM2	WT
Consumption of soil water (mm)	0–1 m	41.3	65.0	59.9	52.5	51.4	62.4	66.7
	1–2 m	74.5	77.5	76.6	70.2	79.0	78.7	84.2
	2–3 m	8.6	23.1	13.7	22.0	17.2	21.1	23.9
	0–3 m	124	166	150	145	148	162	175
Total water consumption (mm)		366	392	408	387	390	404	417
Grain yield (kg/ha)		2,775	3,986	4,299	3,734	4,562	3,995	4,247
WUE (kg/ha/mm)		7.60	10.2	10.5	9.70	11.7	9.90	10.2

TABLE IV. FATE OF LABELED UREA N (% OF APPLIED N)

Treatment	Plant recovery	Recovery in soil				Total recovery	Unaccounted for
		0–10 cm	10–20 cm	20–40 cm	0–40 cm		
N1	38.4a <sup>a</sup>	22.0	5.2	4.6	31.8a	70.2a	29.8
N1O	36.9a	22.1	6.6	4.9	33.6a	70.5a	29.5
N2	36.6a	20.7	4.2	4.3	29.2a	65.8a	34.2

<sup>a</sup>Means within a column followed by the same letter are not significantly different ( $P=0.05$ ) by Duncan's multiple-range test.

TABLE V. COMPARISON OF THE FATE OF UREA N AS BASAL DRESSING ON WINTER WHEAT IN OTHER SOILS

Soil	Application method	Application rate (kg N/ha)	Plant recovery (%)	Recovery in soil (%)	Total recovery (%)	Source
Heilu	BP <sup>a</sup>	100	38	32	70	This study
	BP	150	37	29	66	
Yellow Chao	FI <sup>b</sup>	180	45	26	71	[6]
Chao	BA <sup>c</sup>	75	39	23	62	[7]
Sandy	BP	100	43	21	64	[8]

<sup>a</sup>Broadcast followed by ploughing. <sup>b</sup>Fertilization followed by irrigation. <sup>c</sup>Band application.

### 3.4. Fate of applied urea-N

The fate of the at-planting applied urea-N in the Heilu soil-wheat system is shown in Table IV. Recovery by the wheat in treatments N1, N2 and N1O was 37 to 38%, with the N remaining in the soil (0–40 cm) was in the range 29 to 34%. Thus, the total recovery of the applied N ranged from 66 to 72%, and the fraction of N unaccounted for was in the range of 29 to 34%. There were no significant differences among the treatment means for plant recovery, soil recovery, or total recovery, indicating that the fate of urea-N was not affected by the rate of application or by the use of organic manure. Table IV also shows that when urea was applied as a basal dressing the fertilizer N remaining in the soil was located mostly in the 0- to 10-cm layer and gradually decreased with depth.

TABLE VI. UREA NITROGEN TAKEN UP BY WINTER WHEAT

Treatment	N taken up from urea (mg/plot)		Total N taken up by plant (mg/plot)		Proportion of N from urea (%)	
	Aerial parts	Total plant	Aerial parts	Total plant	Aerial parts	Total plant
N1	690	768	2,076	2,302	33.2	33.3
N2	968	1,097	2,435	2,713	39.8	40.4
N1O	657	738	2,015	2,245	32.6	32.9

The fate of applied N may be affected by factors such as crop species and cultivar, type of soil, and rate, method and timing of fertilizer application [5]. Table V compares the present results with others obtained with urea as a basal dressing applied to winter wheat at other locations on other types of soil, with irrigation. It shows that the fate of applied N in the present rain-fed winter wheat was comparable with the other results obtained with irrigated winter wheat.

Table VI shows no difference between the N1 and N1O treatments for total N taken up by wheat, the amount of N taken up from urea, or the proportion of N taken up from urea, consistent with the observation of no yield increase of N1O compared with N1. In the case of treatment N2, these values apparently increased (Table VI). Whether the proportion of N taken up by wheat from urea was calculated from total-plant data (straw plus roots) or from aerial parts (leaves and stems) the pattern was the same. In treatments N1 and N1O, 33% of N taken up by wheat was derived from urea, whereas 40% was derived from urea for treatment N2. These results are similar to others obtained in China [9]. It is concluded that around a third of the total N taken up by wheat is derived from N fertilizer and the remaining two thirds is derived from the soil. Therefore, improving soil fertility is most important for increasing wheat grain yield.

#### 4. CONCLUSION

Results from the field experiment showed that rational fertilization is an effective management practice to increase the efficiency of utilization of water and nutrients in a rain-fed farming system in the Changwu region. The efficiency of use of urea at a rate of 100 kg N/ha with or without organic manure was good, and an application rate of 150 kg N /ha as urea was excessive in this region for wheat in a dry year. But, to determine what should be recommended to farmers over the long run, data for several seasons are needed in conjunction with a crop model to determine the probability of yield responses due to various specific factors.

Mulching, a management practice for improving water infiltration and reducing soil-water evaporation, has been successfully adopted for maize production in this region; however, it did not show any effect on wheat yield. This merits further testing

Water use efficiency (WUE) was the lowest (7.6 kg/ha/mm) for CK and it increased to 9.70 to 11.7 kg/ha/mm with application of N. The highest WUE was shown in a treatment with mulching.

Results of the <sup>15</sup>N study showed plant recoveries of urea N of approximately 38% for all three treatments, with 29 to 34% remaining in the 0- to 40-cm soil layer. The proportion of unaccounted-for applied N also ranged from 29 to 34%. These are similar to data obtained with irrigated winter wheat in China.

#### ACKNOWLEDGEMENTS

We thank the technicians in FAO/IAEA Agriculture and Biotechnology Laboratory for their analysis for total N and <sup>15</sup>N. This study was funded by IAEA (302-D1-CRP-9986), National project (96-004-05) and the Chinese Academy of Sciences (KZ951-A1-301).



## REFERENCES

- [1] DANG, T.H., et al., Analysis on unusual property of fertilization effect upon winter wheat on arid plain in the worst drought year, *Bulletin Soil Water Con.* **13** (1993) 54–57.
- [2] CHINESE AGRICULTURAL ACADEMY OF SCIENCE, *Chemical Fertilizers in China and Their Distribution*, Agricultural Science Publishing House, Beijing (1986).
- [3] DANG, T.H., Effects of fertilization on water use efficiency of winter wheat in arid highland, *Eco-agric. Res.* **7** (1999) 28–31.
- [4] ZHOU, L.Y., Water supply and productivity in rainfed wheat field in Fengqiu region, *Acta Pedol. Sinica* **30** (1993) 297–303.
- [5] CAI, G.X., FAN X.H., “Nitrogen in soils and efficient use of nitrogen fertilizers”, *Principle of Soil-Plant Nutrition and Fertilization* (LU, R.K., Ed.), Chemical Industry Publisher, Beijing (1998) 112–151.
- [6] ZHANG, X., et al., A study on recommended systems for fertilizing winter wheat in medium and low productive region with  $^{15}\text{N}$  technique, *Soil* **30** (1999) 224–226.
- [7] ZHANG, S.L., et al., The transformation of urea and the fate of fertilizer nitrogen in Fluvo-aquic soil-winter wheat system in flooded plain of Huanghe river, *Acta Agric. Nucleatae Sinica* **3** (1989) 91–5.
- [8] ZHANG, Q.G., et al., Effect of phosphorus fertilizer application on growth and N utilization of winter wheat in fine sandy soil, *Acta Agric. Nucleatae Sinica* **8** (1994) 159–166.
- [9] ZHU, Z.L., et al., Fate and management of fertilizer nitrogen in agro-ecosystems”, *Nitrogen in soils of China* (ZHU, Z.L., et al., Eds.), Kluwer Academic Publishers, Dordrecht (1997) 239–280.

## FATE OF FERTILIZER NITROGEN APPLIED TO WHEAT IN THE MEDITERRANEAN REGION

C. KIRDA, R. DERICI  
Faculty of Agriculture,  
Cukurova University,  
Adana, Turkey

### Abstract

The utilization of fertilizer N by wheat and the amount of applied N residual in the soil and its subsequent recovery by maize and cotton were assessed using  $^{15}\text{N}$  in a 4-year field experiment. Fertilizer, in the range of 0 to 240 kg N ha<sup>-1</sup>, was applied in two parts, a third at emergence and two thirds at tillering. Fertilizer-N recovery by the wheat was two- to three-fold higher with the second split. At physiological maturity, recovery rates of 50 to 60% were obtained for the highest N rate used, suggesting that significant amounts remained in the soil. Although they were supplied with 80 kg N ha<sup>-1</sup>, maize and cotton showed positive yield responses to preceding N treatments to wheat. Recoveries of residual N by maize and cotton were 11 to 15% and over 25%, respectively, of total fertilizer-N applied to the preceding wheat crop. Between 78 and 97% of the total fertilizer N applied to the wheat was accounted for as plant uptake plus residual in the soil. The results, therefore, suggest that volatilization and leaching losses of the applied fertilizer N were small. Residual fertilizer N in the soil, after harvest, was confined mainly to the top 40 cm of the profile, therefore, the risk of leaching fertilizer N during the growing season was essentially nil for this rain-fed wheat grown on a heavy textured soil (Palexerollic Chromoxeret) in the Mediterranean region.

### 1. INTRODUCTION

The fate of fertilizer N applied to soils under a variety of cropping systems has been the subject of considerable research since awareness of environmental pollution and the concept of sustainable agriculture became widespread. Along with other cereals such as maize and rice, wheat varieties are now available that produce high yields in response to elevated levels of fertilizers. However, the fertilizer N recovered by these crops usually is considerably less than that applied. The portion of N not recovered by a crop is prone to loss from the rooting zone via volatilization, denitrification, and leaching, with undesirable ecological and economic consequences.

Recovery of N under increasing levels of fertilizer input for various soil and climate conditions has been investigated in many soils [1–7]. It is evident that a close relationship exists between N utilization and the regional water regime. Lopez-Bellido et al. [6] reported that wheat showed no response to N fertilization if rainfall during the growing season was less than 450 mm. Garabet et al. [4] found that higher rainfall and supplemental irrigation increased N use efficiency (NUE) by wheat while resulting in minimal N losses. Timing of N applications to match plant uptake and utilization during various stages of growth is another important aspect of optimizing NUE. The effects of split applications of N to wheat in the autumn (pre-plant) and the spring (at anthesis) on fertilizer N use efficiency [1,3] and on N losses [3] have been studied in detail. The experimental evidence leads to the conclusion that splitting the applications of fertilizer increases N fertilizer recovery (NFR) under a wide range of conditions.

The primary objective of this work was to determine the fate of fertilizer N applied to wheat in the Mediterranean region by measuring and comparing recoveries of N applied at crop establishment in the autumn and at the tillering in spring, through the use of  $^{15}\text{N}$ . The fate of the fertilizer N left within the soil profile and its recovery by subsequent crops of maize and cotton were also investigated.

## 2. METHODS AND MATERIALS

A 4-year randomized complete block experiment with four replications was laid out in the research fields of Faculty of Agriculture, Cukurova University, Adana, Turkey. The soil is a Palexerollic Chromoxeret with significant amounts of smectite-type clay minerals. Bread-wheat variety Seri 82, popular in the region, was planted at a rate of 220 kg ha<sup>-1</sup> each year, rotating at different sites on the research field. Fertilizer treatments consisted of four rates of N, 0, 80, 160, and 240 kg N ha<sup>-1</sup>, as urea, split in two parts, one third at emergence and two thirds at tillering.

Isotope sub-plots of <sup>15</sup>N were established on the plots receiving 160 and 240 kg N ha<sup>-1</sup>. A uniform enrichment of 5% <sup>15</sup>N-atom excess was used at both rates to facilitate <sup>15</sup>N/<sup>14</sup>N ratio analysis in soil samples as well as in plants. Isotope sub-plots contained six 1.5-m-long rows of wheat, with 15-cm row spacing, amounting to 1.15 m<sup>2</sup> of plot area. Plant and soil samples, at physiological maturity, were collected from the centres of the isotope subplots for <sup>15</sup>N analyses.

Maize ('TTM 815') and cotton ('Cukurova 1518'), as subsequent crops, were planted in 1994/95 and 1995/96 on all plots with a uniform rate of 80 kg N·ha<sup>-1</sup>. The amounts of fertilizer N derived from that applied to the preceding wheat were calculated using the <sup>15</sup>N technique described by Kirda et al. [8].

## 3. RESULTS AND DISCUSSION

In general, there were good biomass and wheat-grain-yield responses to fertilizer-N application in all 4 years. However, the grain-yield responses to similar N rates were highly variable from year to year possibly due to climatic variability and to initial soil N levels (Fig. 1). Lower grain yields (3.0 to 4.0 t ha<sup>-1</sup>) attained during the initial 2 years of the experiment, were due to regionally endemic yellow-rust infections (*Puccinia striiformis* or *P. glumarum*). The largest grain-yield increases were consistently achieved within the range from 0 to 100 kg N·ha<sup>-1</sup>, and the highest yields were consistently obtained with 160 kg N·ha<sup>-1</sup> (Fig. 1).

The wheat benefited most from fertilizer N applied at tillering (Fig. 2). Percent N-fertilizer recoveries (%NFRs) were two- to three-fold higher with the two-thirds application at tillering (Zadoks Scale 2.5 [9]), than with the one third at emergence. There was a similar trend for percentage of plant N derived from fertilizer (%Ndff) (Fig. 2). This yield-independent measure of N fertilizer uptake was consistently higher with the two-thirds split both for 160 and for 240 kg N·ha<sup>-1</sup>, consistent with earlier findings. Olson and Kurtz [10] suggested the practice of latest possible application of N, in accordance with the stage of development, in order to obtain maximum use efficiency of fertilizer. Results obtained in much research work suggest splitting fertilizer-N application, leaving the greater portion for the later time. Garabet [4] reported that fertilizer recovery is not uniform; it increases through the season and reaches a maximum near anthesis.

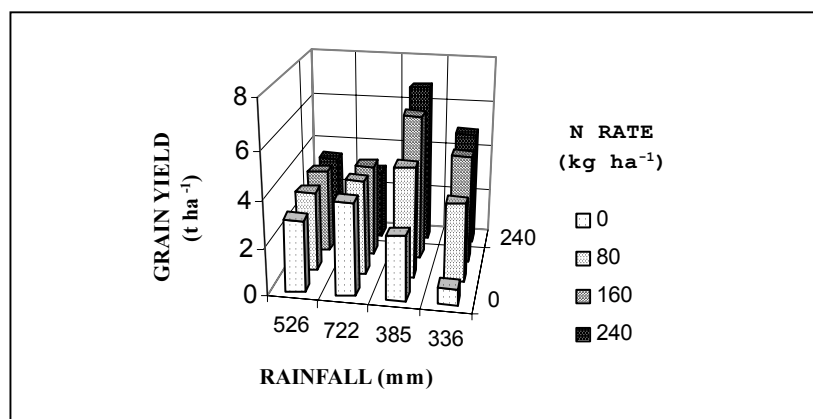


FIG. 1. Wheat yield response to N fertilizer.

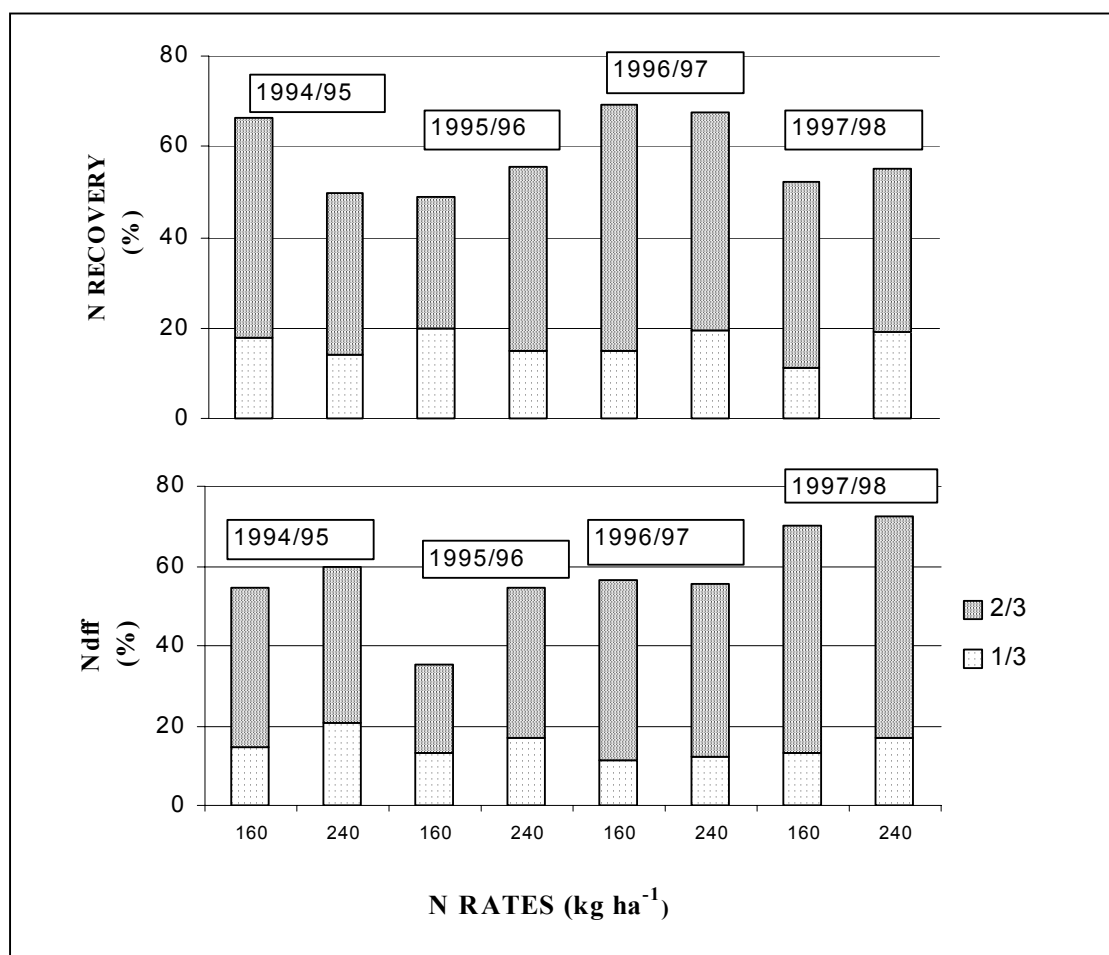


FIG. 2. Fertilizer N recovery (top) and the %N derived from fertilizer (bottom) values under two split fertilizer applications over 4 years.

Applications of high rates at early stages of growth do not necessarily guarantee maintenance of adequate levels of N. Wuest and Cassman [2], working on wheat, reported that the rate of preplant-applied N did not significantly affect plant-available N in the soil at anthesis, and both extractable and mineralizable N contents of soil were similar with preplant applications of 120 and 240 kg N ha<sup>-1</sup>. Furthermore, estimated amounts of soil N taken up by wheat were not affected by the time of application. Fertilizer-N recoveries for the 3-year period were 41 and 68% for preplant and anthesis applications, respectively. Therefore, they recommended the maintenance of high levels of N in the soil at anthesis to attain high levels both of grain yield and of grain N content. With respect to timing of N application, Mahler et al. [1] concluded from their 4-year experiment on winter wheat in a region receiving 480 to 650 mm precipitation, that 75% of the total N should be applied in the spring for higher yields and improved N use efficiency. The results of this work, along with evidence offered in other reports, therefore, suggest that in the coastal areas of the Mediterranean region where it rains usually from November until March or April, wheat benefits more from N applied at tillering as compared with earlier application near planting. Furthermore, depending on the preplant N content of the soil, the proportion of the first split may be lowered below the present practice of one third of the total, leaving the higher proportion for the application at tillering.

The amount of fertilizer N left in the soil after the wheat harvest in 1997 was higher close to the surface and decreased with depth, reaching scarcely detectable values ( $\leq 20$  mg N m<sup>-2</sup> cm<sup>-1</sup>) beyond 60 cm (Fig. 3). Data for other years (not shown) followed this trend. The results therefore show that leaching of N fertilizer below 60 cm is not likely. It can, therefore, be concluded that risks of leaching of N fertilizer are low under the local farmers' general practice of applying 160 kg N ha<sup>-1</sup> to wheat.

Further work is needed, however, to examine transformations, e.g. immobilization and mineralization of fertilizer N within the soil profile, that may contribute to leaching in subsequent years.

In Mediterranean conditions, previously reported %NFR values have shown a wide range of variability, 9 to 75% [4,7,11,12], possibly reflecting specific differences in soil fertility and climate variations from year to year. It is, therefore, expected that a significant fraction of fertilizer applied to wheat may remain in the soil after harvest. In this work, the range of recovery of applied fertilizer N was 50 to 70% with N application rates of 160 and 240 kg N ha<sup>-1</sup> (Fig. 2). The remaining fraction (30 to 50%) was retained within the soil profile after harvest (Table I). Thus, yields of subsequent maize and cotton, although they received 80 kg N ha<sup>-1</sup>, showed strong responses to N applied to wheat (Table II). Recovery of residual N by maize and cotton was over 10 to 15% and over 25% of total N applied, respectively (Table II).

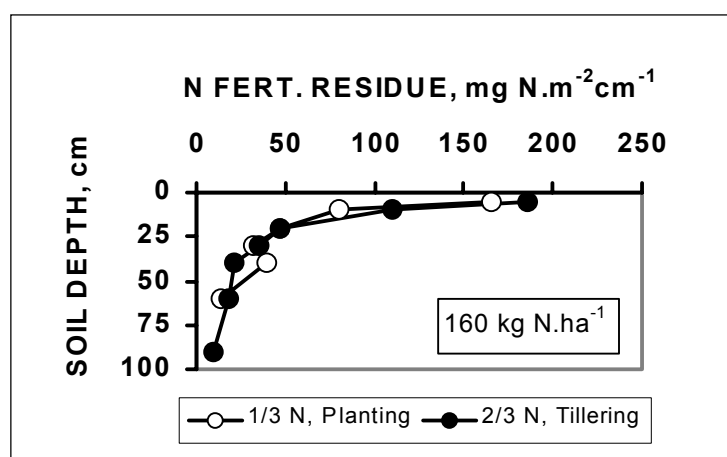


FIG. 3. Fertilizer N residual in the soil after wheat harvest in 1997.

TABLE I. PLANT RECOVERY AND MEASURED RESIDUAL IN SOIL OF FERTILIZER N APPLIED TO WHEAT AT 240 kg N ha<sup>-1</sup>

N application	Plant uptake (kg N ha <sup>-1</sup> )	Residual in soil <sup>a</sup> (kg N ha <sup>-1</sup> )	Unaccounted fertilizer (%)
1994–95			
1/3 80 kg N ha <sup>-1</sup>	42.8	35.3	
2/3 160 kg N ha <sup>-1</sup>	88.0	81.8	≈0.0
1995–96			
1/3 80 kg N ha <sup>-1</sup>	37.7	32.2	
2 /3 160 kg N ha <sup>-1</sup>	104	14.1	22
1996–97			
1/3 80 kg N ha <sup>-1</sup>	47.0	24.3	
2/3 160 kg N ha <sup>-1</sup>	115	46.8	2.8
1997–98			
1/3 80 kg N ha <sup>-1</sup>	31.6	50.1	
2/3 160 kg N ha <sup>-1</sup>	86.4	61.2	4.5

<sup>a</sup>Calculated based on N fertilizer distribution profiles like Fig. 3, measured through soil sampling, immediately after the harvest of the wheat crop.

TABLE II. YIELDS AND RECOVERY OF RESIDUAL N FERTILIZER BY SECOND CROP MAIZE AND COTTON, PLANTED IN ALTERNATING YEARS SUCCEEDING WHEAT

Component	N treatment to the preceding wheat			
	N <sub>0</sub> 0 kg N ha <sup>-1</sup>	N <sub>1</sub> 80 kg N·ha <sup>-1</sup>	N <sub>2</sub> 160 kg N·ha <sup>-1</sup>	N <sub>3</sub> 240 kg N·ha <sup>-1</sup>
Second crop maize, summer 1995				
Grain yield, t ha <sup>-1</sup>	8.4	8.7	10.3	12.7
Kernel, kg N ha <sup>-1</sup>		5.2	12.5	21.7
Vegetative parts, kg N ha <sup>-1</sup>		4.0	7.8	14.5
Total, kg N ha <sup>-1</sup>		9.2	20.3	36.2
% of total N applied		11	13	15
% of residual N		—	25	34
Second crop cotton, summer 1996				
Cotton yield, t·ha <sup>-1</sup>	3.8	5.2	5.0	4.6
Total, kg N·ha <sup>-1</sup>		22.0	35.2	68.0
% of total N applied		27	22	28
% of residual N		—	27	26

#### 4. CONCLUSIONS

Nitrogen-application practices with wheat crops in Mediterranean coastal regions require a thorough evaluation, in terms both of rates and of timing. The rate of 160 kg N ha<sup>-1</sup>, commonly used by farmers, seems to be adequate, especially if soil-N level is not very low. However, the present split practice should be modified by applying less than one third at planting, since the N applied at tillering was better utilized and left proportionately less N in the soil.

Residual N in the soil after harvest of the wheat, was confined to the surface 40 cm; therefore, the risk of leaching fertilizer N during the growing season was essentially nil. Subsequent crops of maize and cotton may assimilate up to 30%, of residual fertilizer N from a preceding crop of wheat. If rates in the range of 160 to 200 kg N ha<sup>-1</sup> are used for wheat, and if, additionally, a second crop follows wheat, risks of leaching fertilizer N may not exist.

#### ACKNOWLEDGEMENTS

We thank the International Atomic Energy Agency (IAEA), Vienna, for financial support, through research contract TUR/7947. Thanks are also due to the technical staff at IAEA's laboratory at Seibersdorf, Austria, for <sup>15</sup>N analyses. The authors are also grateful for partial support received from the C.U. Faculty of Agriculture, Adana, through research grant BAP-TYS-95/08.

#### REFERENCES

- [1] MAHLER, R.L. et al., Nitrogen source, timing of application and placement: Effects on winter wheat production, *Agron. J.* **86** (1994) 637–642.
- [2] WUEST, S.B., CASSMAN, K.G., Fertilizer-nitrogen use efficiency of irrigated wheat: I. Uptake efficiency of preplant versus late season application, *Agron. J.* **84** (1992) 682–688.
- [3] ALCOZ, M.M., et al., Nitrogen fertilisation, timing effects on wheat production, nitrogen uptake efficiency, and residual soil nitrogen, *Agron. J.* **85** (1993) 1198–1203.

- [4] GARABET, S., et al., "Field estimates of nitrogen-use efficiency by irrigated and rainfed wheat in a Mediterranean climate", *Accomplishments and Future Challenges in Dryland Soil Fertility Research in the Mediterranean Area* (RYAN, J., Ed.), ICARDA, Aleppo (1997) 95–106.
- [5] PALA, M., "Use of models to enhance nitrogen use by wheat", *Accomplishments and Future Challenges in Dryland Soil Fertility Research in the Mediterranean Area* (RYAN, J., Ed.), ICARDA, Aleppo (1997) 135–144
- [6] LOPEZ-BELLIDO, K., et al., Long term tillage, crop rotation and nitrogen fertilizer effects on wheat yield under rainfed Mediterranean conditions, *Agron. J.* **88** (1996) 783–791.
- [7] WOOD, M., et al., "Nitrogen cycling in a dryland cereal-legume rotation system", *Accomplishments and Future Challenges in Dryland Soil Fertility Research in the Mediterranean Area* (RYAN, J., Ed.), ICARDA, Aleppo (1997) 71–78.
- [8] KIRDA, C., et al., "Plant nutrient and water balance studies under legume-cereal rotation systems", *Nuclear Methods for Plant Nutrients and Water Balance Studies*, IAEA-TECDOC-875 (1996) 11–22.
- [9] ZADOKS, J.C., et al., A decimal code for the growth stages of cereals, *Weed Res.* **14** (1974) 415–421.
- [10] OLSON, R.A., KURTZ, L.T., "Crop N requirements, utilization and fertilization", *Nitrogen in Agricultural Soils*. (STEVENSON, F.J., Ed.), ASA, CSSA and SSSA, Madison (1982) 567–604.
- [11] ABDEL-MONEM, M., RYAN, J., "Nitrogen use efficiency in WANA determined by <sup>15</sup>N technique", *Accomplishments and Future Challenges in Dryland Soil Fertility Research in the Mediterranean Area* (RYAN, J., Ed.), ICARDA, Aleppo (1997) 57–63.
- [12] SFARY, F., CHARANEK, A., "Nitrogen fertilizer-use efficiency studies by Syrian Atomic Energy Commission using <sup>15</sup>N labeled fertilizers", *Accomplishments and Future Challenges in Dryland Soil Fertility Research in the Mediterranean Area* (RYAN, J., Ed.), ICARDA, Aleppo (1997) 64–70.

## NITROGEN BALANCE IN IRRIGATED POTATOES IN SANDY-TEXTURED SOILS\*

M.B. HALİTLİGİL, A. AKIN

Ankara Nuclear Research and Training Centre,  
Turkish Atomic Energy Authority

A. İLBİYİ

Rural Affairs Research Institute,  
General Directory of Rural Affairs,

Ankara, Turkey

### Abstract

To obtain information on potato yield, N uptake, residual fertilizer N in the soil, and the portion of fertilizer N leached below a depth of 200 cm, field experiments were conducted at three locations in 1992, 1993, and 1994, with soil textures ranging from sandy to silty loam in the Cappadocia region of Turkey. In all of the experiments ammonium sulphate was applied at six rates (0, 200, 400, 600, 800, and 1,000 kg N/ha). The fate of the applied fertilizer N was determined for the 400 and 1,000 kg N/ha rates by using ammonium sulphate enriched in  $^{15}\text{N}$  at 5 and 2% atom excess, respectively. Sprinkler irrigation was applied weekly, starting after hilling in mid-June. Irrigations ranged from eleven to seventeen depending on year and location. Optimum marketable tuber yields were obtained with 600 kg N/ha. The amount of fertilizer N in the 0- to 200-cm soil layer increased more than three fold when the N rate was increased from 400 to 1,000 kg/ha. Nearly half of the applied fertilizer N (46%) at 400 kg/ha and more than half of it (61%) at 1,000 kg/ha remained in the 0- to 200-cm soil layer after harvest. Four times more N fertilizer was leached beyond a depth of 200 cm when 1,000 kg N/ha was applied in comparison with 400 kg N/ha.

### 1. INTRODUCTION

Nitrogen is the nutrient that most commonly limits crop yields. Therefore, N fertilizers are being consumed extensively all over the world. The efficiency of their uptake by plants (20 to 60% for cereals, 40 to 80% for vegetables and grasses) is affected by the soil, climate, and management conditions [1]. The non-assimilated N either remains in the soil profile or is lost by volatilization, denitrification, and leaching to surface and ground waters.

Although frequent irrigation and high fertilizer-N application can dramatically increase yields of potato (*Solanum tuberosum* L.), leaching of N can cause  $\text{NO}_3^-$  pollution that is hazardous to the health of humans and animals [2–4]. In one study, most of the  $^{15}\text{N}$ -labelled nitrate movement occurred in the 0- to 160-cm soil layer after the first year of application [2], and nitrate concentrations of well waters near heavily fertilized farmlands increased from 5 to 35 mg  $\text{L}^{-1}$  in 3 years. Due to their light-textured soils, nearly 40,000 ha [5,6] of the farmed lands of the Derinkuyu, Suvermez, and Kuyulutlar regions of the Nevşehir province of Central Anatolia, Turkey, are used mainly for potato cultivation. Recently, farmers began to use new high-yielding varieties to which they apply very high amounts of N fertilizers—sometimes more than 900 kg N/ha—and, frequently, high irrigation rates are used in order to optimize yields. Some researchers [10] have conducted fertilizer-N trials with potatoes in this area. However, the aim of those investigations was only to document responses to several N rates and no data were generated concerning the fate of the applied N, the amount taken up by the potato crop or, especially, the unrecovered N residue in the soil profile or the leached below 200 cm. Therefore, the main objectives of this study were:

- to investigate the marketable potato tuber yield / N rate relationship in sandy textured soils,
- to document the fate of  $^{15}\text{N}$ -labelled ammonium sulphate fertilizer (in the potato crop, in the soil, and the portion lost or leached) applied at 400 and 1,000 kg N/ha.



## 2. MATERIAL AND METHODS

Nine field experiments were conducted in the province of Nevşehir, at Merkez, Suvermez and Kuyulutatlar in 1992, at Suvermez and two locations at Kuyulutatlar in 1993, and at Merkez and two locations at Kuyulutatlar in 1994. Nevşehir is in the Cappadocia region of the Central Anatolian Plateau. Mean annual rainfall is about 410 mm, most of which falls in winter and spring. All experiments were carried out on typically sandy-textured soils belonging to the Regosol great soil group. They are low in organic matter content and have poor water-retention properties, are low in P content, high in K, neutral in pH, and have no salinity or drainage problems (Table I).

The experimental design used in all experiments was a completely randomized block with three replications. The N rates were 0, 200, 400, 600, 800, and 1,000 kg N/ha as  $(\text{NH}_4)_2\text{SO}_4$ , applied in furrows in equal portions at planting and immediately prior to the first irrigation. Triple superphosphate was applied to all plots at 100 kg  $\text{P}_2\text{O}_5$ /ha, at seed-bed preparation. Each plot measured 4.2×5.1 m (21.4 m<sup>2</sup>) with 70 and 50 cm between- and within-row spacings, respectively. The high cost of <sup>15</sup>N-labelled fertilizer and differences in recommendations of N rate for potato, as well as the N rate local farmers generally apply, dictated the use of isotope microplots (0.9×1.4 m = 1.26 m<sup>2</sup>) only for 400 and 1,000 kg N/ha, for which 5.0 and 2.5% <sup>15</sup>N a.e. enrichments were used, respectively. Each isotope microplot contained six plants. Nitrogen-15-labelled fertilizer, in granular form, was also applied in furrows, half at planting and the other immediately before the first irrigation.

Eleven to seventeen irrigations were made (Table II) with sprinklers, the method used exclusively in the region. At the end of September or beginning of October, plants from each plot (2.8×3.6 m = 10.1 m<sup>2</sup>) were harvested and separated into tuber and leaf plus vine. Marketable tuber and dry matter yields were determined for all N treatments in order to construct a N-response curve. For the <sup>15</sup>N-balance study, tuber and leaf plus vine of two out of six plants from each <sup>15</sup>N isotope-subplot were dried (70°C), ground to pass through a 2-mm screen for %N and %<sup>15</sup>N a.e. analyses by the micro-Kjeldahl method (Keltex) and emission spectrophotometry (Jasco 150), respectively [7]. The N yield (kg/ha), %Ndff, fertilizer-N yield (kg N/ha) and %NUE values were calculated according to [8].

Soil samples from 0 to 200 cm, in 20-cm increments, were taken from each experimental site just before planting, and the total-N contents were determined. Also, soil samples were taken from the same depths of the 0, 400 and 1,000 kg N/ha rate plots after the potato harvest, and total N,  $\text{NO}_3^-$  and <sup>15</sup>N-labelled  $\text{NO}_3^-$  determinations made in order to estimate residual fertilizer-N. The amounts of fertilizer N leached below 200 cm soil depth (which is the unaccounted-for or lost portion) was calculated at both 400 and 1,000 kg N/ha rates by subtracting the amount of plant-N uptake plus fertilizer-N residue in 200 cm soil depth from the amount of fertilizer-N applied.

Analysis of variance for the marketable tuber yields for each experiment and regression analysis including all the data obtained in the 3 years were done according to [9]. Also, for the <sup>15</sup>N data, the standard deviations for dry matter, N yield, fertilizer N in the 200-cm soil layer, for each treatment at each location and year were calculated. Standard deviations were calculated for the averages over locations and years.

## 3. RESULTS AND DISCUSSION

### 3.1. The marketable tuber yield / N-rate relationship

In all of the trials, the marketable tuber yields were significantly increased ( $P < 0.01$ ) by N fertilization in comparison to the unfertilized treatments. The N rates that produced the highest marketable yields varied from trial to trial. From the nine trials, the highest tuber yields were obtained with three 400-kg N/ha rates, four 600-kg N/ha rates and one 200-kg N/ha rate. In only one experiment was there no significant difference in tuber yield among the N rates. The response curve of marketable tuber yield to increasing rates of fertilizer N is shown in Fig. 1.

TABLE I. PROPERTIES OF THE SOILS (0–20 cm) AT THE EXPERIMENTAL SITES

Year and location	Texture	pH	Salt (%)	CaCO <sub>3</sub> (%)	OM (%)	P <sub>2</sub> O (kg/ha)	K <sub>2</sub> O (kg/ha)
1992 Merkez Suvermez Kuyulutlar	Sandy Loamy sand Loamy sand	6.5 6.9 6.2	0 0.01 0	0 0 0	0.46 0.92 0.92	102 203 249	690 1,825 1,217
1993 Kuyulutlar 1 Suvermez Kuyulutlar 2	Loamy sand Silty loam Silty loam	6.6 6.9 6.8	0.01 0.05 0.07	0 0 0	0.29 0.34 0.29	60.9 142 130	845 1,591 1,556
1994 Merkez Kuyulutlar 1 Kuyulutlar 2	Silty loam Loamy sand Silty loam	6.9 7.2 6.9	0.03 0.03 0.04	0 0 0.03	0.46 0.29 0.34	44.3 19.9 64.2	7,956 450 936

TABLE II. DATES OF AGRICULTURAL PRACTICES

Location	1992		1993		1994	
	Merkez	Suvermez	Kuyu. 1	Suvermez	Kuyu. 1	Kuyu. 2
Potato Variety	Granola	Marfona	Marfona	Granola	Granola	Granola
Planting	23.04.92	28.04.92	29.04.92	30.05.93	11.05.94	11.05.94
First N appl'n	23.04.92	28.04.92	29.04.92	01.06.93	11.05.94	11.05.94
Second N appl'n	18.06.92	18.06.92	18.06.92	06.07.93	14.06.94	15.06.94
First irrigation	19.06.92	19.06.92	19.07.92	07.07.93	15.07.94	16.07.94
Irrigation number <sup>a</sup>	17	11	13	14	13	13
Manual weed control	27.06.92	27.06.92	22.06.92	09.07.93	24.07.94	18.07.94
Pesticide appl'n <sup>b</sup>	03.06.92	07.07.92	07.07.92	02.07.93	20.07.94	20.07.94
Harvest	24.09.92	23.09.92	24.09.92	13.10.93	29.09.94	28.09.94

Each irrigation was about 100 mm of water by sprinkler. <sup>b</sup>For potato bug and weeds.

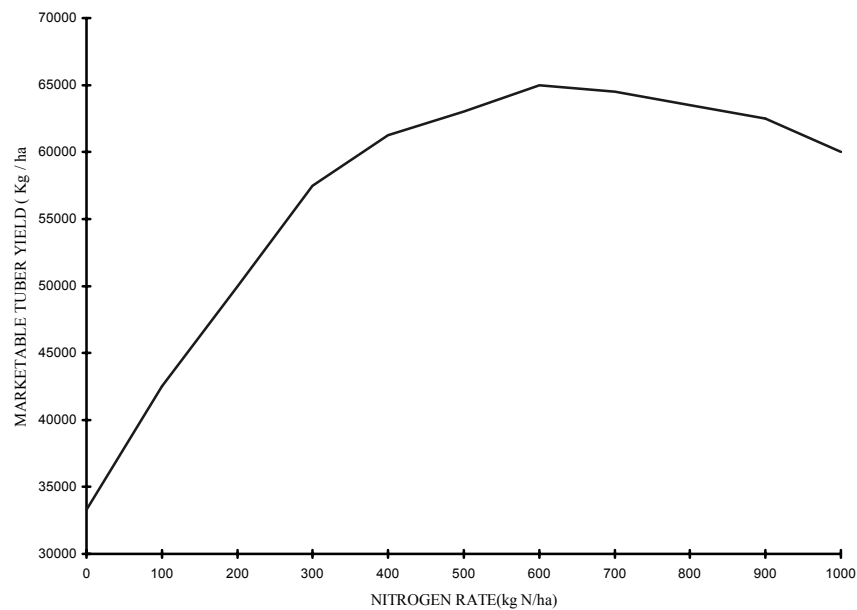


FIG. 1. Relationship between marketable potato tuber yield and N fertilization.

Regression analysis was used to find the marketable tuber yield / N relationship. The curve obtained can be described by the equation:

$$Y = 3329.9 + 101.7X - 0.747X^2$$

where

Y is the yield (kg/ha),  
and X is the N rate (kg/ha).

For dry tuber yield the following relationship was obtained:

$$Y = 585.16 + 14.62 X - 0.12X^2$$

From these two equations, the amount of N fertilizer to be applied for optimum potato tuber yields under our experimental conditions was around 600 kg N/ha. This is very high amount of N can be explained only in terms of the frequent (almost every 5 to 7 days) high rates (100 mm) of sprinkler irrigation. Similar results have been obtained from other investigations conducted in the same region [10]: optimum marketable tuber yields were obtained with 600 kg N/ha, although the economical rate was found to be 500 kg N/ha. Our results clearly indicate that N rates above 600 kg N/ha would be uneconomical with respect to optimum marketable tuber yields. Research in another country [11] on sandy to loamy sandy soils reported that optimum potato yields and the most economic returns were obtained with 340 kg N/ha.

### 3.2. Nitrogen-15 balance

When averages over locations and years are considered, tuber and total dry matter yields showed slight decreases with 400 to 1,000 kg N/ha, whereas leaf plus vine dry matter yields showed slight increases (Table III). Also, tuber N accumulation was increased slightly, and leaf plus vine N assimilation increased considerably as the N rate was increased. These results are consistent with another report [11] that excessive N applied to potatoes shows up as increased N in the shoots especially in the vines.

When averaged over year and location, %Ndff increased from 60 to 67, while the Ndff (kg N/ha) increased from 174 to 211, as N rate increased from 400 to 1,000 kg N/ha, respectively. Also N uptake from the soil N pool increased with fertilization (110 and 116 kg N/ha for 0 and 400 kg N/ha rate, respectively) beyond which increased rates of applied N rate resulted in less N uptake from the soil (103 kg N/ha with 1,000 kg N/ha).

Averaged %NUE values decreased from 42 to 21 and the N fertilizer residue in the 0- to 200-cm soil depth increased from 182 to 608 kg N/ha, respectively, with increasing N rate. We obtained low %NUE values, which is to be expected with irrigated sandy soils [2, 11–14]. Under such soil and management conditions, ways of increasing the efficiency of N use must be investigated; one possibility is to make several applications of N fertilizer during the growing period [11].

Our data show that almost half of the applied N fertilizer (46%) at 400 kg N/ha and more than half of it (61%) at 1,000 kg N/ha remained in the 0- to 200-cm soil depth after harvest. Also, the amount of <sup>15</sup>N-labelled NO<sub>3</sub><sup>-</sup> found was two-fold higher at 1,000 than at 400 kg N/ha for each 20-cm increment of the 0 to 200 cm soil depth (Fig. 2). This trend was consistent across locations and years.

Due to rapid nitrification in well aerated sandy soils, there is a potential for leaching of nitrate. In our study, the amount of fertilizer N leached below 200 cm was not measured. It was calculated and, therefore, standard deviation values were not calculated for them. As can be clearly seen from Table III, under our experimental conditions, much more fertilizer N (four-fold) was leached beyond 200 cm soil depth when 1,000 kg N/ha nitrogen was applied in comparison to 400 kg N/ha (181 and 44 kg N/ha, respectively).

TABLE III. FERTILIZER N EFFECTS ON DRY MATTER YIELD, N UPTAKE, Ndff (% AND N/ha ), TOTAL N IN THE 0–200 cm LAYER, AND FERTILIZER N RESIDUE IN THE 0–200 cm LAYER, FERTILIZER N LEACHED BELOW 200 cm, AT THREE N RATES AVERAGED OVER YEARS AND LOCATIONS

Component	N applied (kg/ha)		
	0	400	1,000
Dry matter (kg/ha)			
Tuber	5,184(789) <sup>a</sup>	10,423(1,586)	9,024(1,308)
Leaf + vine	2,447(411)	4,511(758)	4,642(733)
Total	7,631(1,147)	14,934(2,245)	13,666(2,023)
N uptake (kg/ha)			
Tuber	70.5(11.2)	201(17.4)	207(16.2)
Leaf + vine	40.0(6.00)	89.0(10.7)	106(11.3)
Total	110(15.2)	290(24.6)	313(25.8)
Ndff (%)	—	60(2.7)	67(3.0)
(kg/ha)	—	174(8.60)	211(9.80)
NUE (%)	—	42(14)	21(7.4)
Fertilizer N residue <sup>b</sup> (kg/ha)	—	182(16.8)	608(21.2)
Fertilizer N leached <sup>c</sup> (kg/ha)	—	44	181

<sup>a</sup> Standard deviation in parentheses. <sup>b</sup> In the 0- to 200-cm soil layer. <sup>c</sup> Below 200 cm.

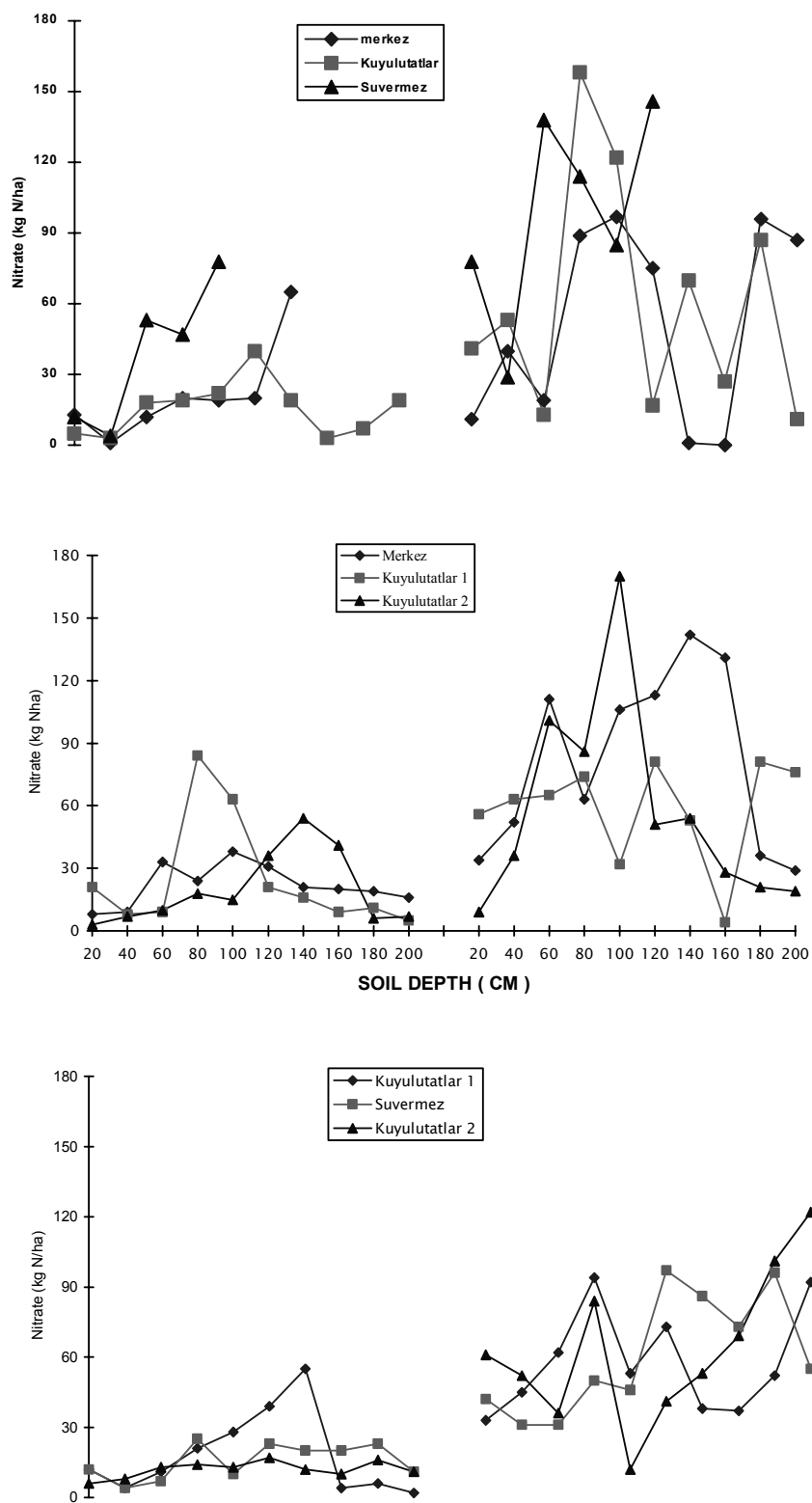


FIG. 2.  $^{15}\text{NO}_3^-$  at 0- to 200-cm soil depths at three locations in 1992 (top), 1993 (middle) and 1994 (bottom) (left=400 kgN/ha, right=1,000 kg/ha).

#### 4. CONCLUSIONS

Our results confirmed that N application by farmers of rates of 900 kg/ha and more to potato crops is unnecessary in the Nevşehir province, for two reasons:

- The optimum marketable tuber yield can be obtained at a maximum of 600 kg N/ha; there is no need for higher N-fertilizer rates.
- With very high N rates, farmers are probably polluting the groundwater. This is important because they are using water from wells for irrigation and drinking that can cause serious health problems in the region in the future.

Nitrogen fertilization-irrigation research in this potato-growing area should continue extensively in order to find ways to increase the N use efficiency. This can be accomplished by applying water and fertilizer N in small amounts and more frequently, especially with drip-irrigation and fertigation practices, which can decrease water usage and increase the %NUE, and thus limit the nitrate movement in the soil.

#### ACKNOWLEDGEMENT

Labelled  $^{15}\text{N}$  fertilizer and the neutron moisture probe were made available by the Soil and Water Management & Crop Nutrition Section of the IAEA through TUR/05/16 technical assistance project. We are grateful for their support.

#### REFERENCES

- [1] PARKER, H., How fertilizer moves and reacts in soil, Crop and Soil Magazine No. 1055 (1972) 21.
- [2] PALTINEANU, I.C., et al., "Irrigation water and N fertilizer application efficiencies for reduction of water and N losses and for water pollution control", Soil Nitrogen as Fertilizer or Pollutant (Proc. Research Coordination Meeting at Piracicaba, Brazil) IAEA, Vienna (1980) 169–194.
- [3] RAWITZ, E., et al., "Fate of fertilizer nitrogen in irrigated fields under semi-arid conditions", Soil Nitrogen as Fertilizer or Pollutant (Proc. Research Coordination Meeting at Piracicaba, Brazil) IAEA, Vienna (1980) 195–236.
- [4] FILIPOVIC, R., STENANOVIĆ, D., "Soil and water nitrate levels in relation to fertilizer utilization in Yugoslavia", Soil Nitrogen as Fertilizer or Pollutant (Proc. Research Coordination Meeting at Piracicaba, Brazil) IAEA, Vienna (1980) 37–46.
- [5] ANONYMOUS, Nevşehir Province Production Documents and Fertilizer Need Report, Publication No. 776, General Directory of Rural Affairs, Ankara (1987).
- [6] ANONYMOUS, Agricultural Structure and Production, Publication No. 1055, State Statistics Institute, Ankara (1989).
- [7] AXMANN, H., et al., "Sample preparation techniques of biological material for isotope analysis", Use of Nuclear Techniques in Studies of Soil-Plant Relationships (HARDARSON, G., Ed.), IAEA, Vienna (1990) 41–54.
- [8] HALITLIGIL, M.B., et al., Effect of nitrogen fertilization on yield and nitrogen and water use efficiencies of winter wheat (durum and bread ) varieties grown under conditions found in Central Anatolia, Biol. Fertil. Soils **31** (2000) 175–182.
- [9] STEEL, R.G.D., TORRIE, J.H., Principles and Procedures in Statistics, McGraw Hill, New York (1960).
- [10] KARACA, M., DEMİR, Z., "Irrigation and N rate influences to potato tuber yields and inorganic N residue in the soil profile at Cappadocia region", Ilhan Akalan Soil and Environment Symposium, Ankara University Agricultural Faculty Publications, Ankara (1995).
- [11] LAUER, D.A., Nitrogen uptake patterns of potatoes with high-frequency sprinkler-applied N fertilizer, Agron. J. **77** (1985) 193–197.

- [12] KORTE, F., SOTRITION, N., "Balance study of the fate of  $^{15}\text{N}$  fertilizer", Soil Nitrogen as Fertilizer or Pollutant (Proc. Research Coordination Meeting at Piracicaba, Brazil), IAEA, Vienna (1980) 105–126.
- [13] WESTERMANN, D.T., KLEINKOFT, G.E., Nitrogen requirements of potatoes, Agron. J. **77** (1985) 616–621.
- [14] SAFFIGNA, P.G., et al., Nitrogen, chloride and water balance with irrigated Russet Burbank potatoes in central Wisconsin, Agron. J. **69** (1977) 251–257.

## OVERVIEW OF THE IAEA PROGRAMME ON FERTIGATION STUDIES IN THE MEDITERRANEAN REGION

P. MOUTONNET

Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture,  
International Atomic Energy Agency,  
Vienna

L.K. HENG

Soil Science Unit,  
FAO/IAEA Agriculture and Biotechnology Laboratory,  
Seibersdorf, Austria

### Abstract

Water is a scarce resource in Mediterranean countries. The optimal water requirement per capita is estimated to be around 1,700 m<sup>3</sup>/year; however, in many countries in West Asia, the available water is less than 500 m<sup>3</sup>/capita/year. The situation will deteriorate further during the next two decades as populations increase. Agriculture is the biggest consumer. About 80% of the renewable water resources are used for irrigation. Traditional methods such as furrow and surface irrigation are highly inefficient: only a third of the applied water is transpired by the crop. Clearly, there is great need to improve irrigation management. Recognizing the potential usefulness of nuclear techniques in fertigation studies, the IAEA implemented a Regional Technical-Cooperation Project from 1995 to 1998 with scientists in eight participating countries: Cyprus, Iran, Jordan, Lebanon, Saudi Arabia, Syria, Turkey, and the United Arab Emirates. The main objective was to examine water balance and fertigation practices, using nuclear techniques—soil moisture neutron probe and <sup>15</sup>N-labelled fertilizers—with a view to improving crop production in arid and semi-arid zones. The objectives were to compare conventional fertilization methods with N-fertigation, by 1) evaluating the recovery of fertilizer N applied, 2) evaluating water use efficiency and estimating crop water requirements, and 3) evaluating potential nitrate pollution. To achieve these objectives, field experiments were included the following treatments: 1) N<sub>s</sub> = N applied to the soil surface by furrow irrigation at the locally recommended rate, 2) N<sub>0</sub> = control, no N application, with drip irrigation, 3) N<sub>1</sub> = N applied by fertigation at 50% of the locally recommended rate, 4) N<sub>2</sub> = N applied by means of fertigation at 100% of the locally recommended rate, and 5) N<sub>3</sub> = N applied by means of fertigation at 150% of the locally recommended rate. At least four sets of data were collected for each of the countries involved. Crops included: tomato, pepper, potato, cotton, lettuce, garlic, and cucumber. Results clearly showed the superior efficiency of fertigation in terms of water use, fertilizer-N recovery, and crop yields. Summarized data show that fertigation is an efficient technique for conserving water and fertilizer N, and for increasing crop production. On average: 1) 42% of irrigation water was saved under drip irrigation, 2) there was a 42% increase in yield for fertigation compared with traditional fertilizer and water-management practices, 3) there was a 79% increase in efficiency of use of irrigation water based on crop yield.

### 1. INTRODUCTION

Mediterranean countries are severely deficient in water resources for agricultural, municipal, and industrial purposes. This situation is aggravated by rapidly increasing populations. The optimal water requirement per capita is around 1,700 m<sup>3</sup>/year, whereas in West Asia eight out of eighteen countries have less than 500 m<sup>3</sup>/capita/year (Table I). The situation will deteriorate over the next two decades. It is noteworthy that agriculture is the biggest consumer of this scarce resource: about 80% is used for irrigation. Traditional irrigation methods, such as the surface and furrow application, are highly inefficient. Estimates indicate that only about a third of the irrigation water is actually transpired by the crop [1]. Clearly, there is great need to improve irrigation management.

Furthermore, lack of water is hindering economic growth; according to Kemp [2], “The World Bank argues that the allocation of water to agriculture, which accounts for about 90% of regional water use, no longer makes economic sense. . . . In Morocco, for example, it is estimated that the value added by a cubic meter of water in irrigated agriculture is a mere 15 cents, whereas, used in industry, it is a striking \$25. In Jordan, which uses highly efficient drip irrigation for over half of its irrigated agriculture, the



equivalent figures are 30 cents for agriculture and \$15 for industry.” Therefore, there is an urgent need to improve the efficiency of use of irrigation water, inasmuch as high input agriculture associated with the mismanagement of irrigation poses a threat to groundwater quality.”

There are several ways of reaching this objective: conservation, recycling, reuse, and improved water use efficiency are some of the approaches being examined [3]. The drip or microirrigation technique developed in the 1960s has spread rapidly worldwide, especially in Mediterranean countries. This method uses 30 to 50% less water than surface irrigation [4]. In Israel, thanks to improved irrigation management, the crop water use efficiency was more than doubled from 1955 to 1980: 1 to 2.5 kg/m<sup>3</sup> over this period [5].

However, drip irrigation has some disadvantages; one of its main drawbacks is the financial investment required for installation. A comparison with sub-irrigation and seepage for tomato production in Florida indicated that drip irrigation reduced water needs by 50%, but added \$328/ha to yearly production costs [6]. Where advanced technology is not required for the distribution network, a low-cost drip system has been promoted for small farmers of developing countries [7]. Instead of an investment of \$2,000/ha, the cost was reduced by 90% by i) making dripper lines moveable, ii) replacing drippers by simple 0.7-mm holes, and iii) using cloth filters instead of expensive alternatives. The low-cost system was tested in Nepal and India. In the latter country [8] the results showed that investment in drip irrigation is economically feasible even though it is capital intensive.

In Mediterranean countries the impact of fertigation/chemigation on agricultural production was thoroughly reviewed by an Expert Consultation held in Cairo, Egypt, in 1991 [9] sponsored by the Food and Agriculture Organisation (FAO) of the United Nations. General conclusions and recommendations were drawn. It was emphasized that very little fertigation/chemigation research has been conducted in countries of West Asia, and that there is a need to develop multidisciplinary research activities involving international, regional, and national institutions, as well as the private sector.

TABLE I. WATER AVAILABILITY AND WITHDRAWAL IN SOME COUNTRIES OF THE WEST ASIA-NORTH AFRICA REGION (FROM [1])

Country	Renewable resources per capita (m <sup>3</sup> /year)		Share of withdrawals (%)	
	1990	2025	Domestic	Agriculture
Algeria	737	354	22	74
Egypt	1,112	645	7	88
Israel	467	311	16	79
Jordan	224	91	29	65
Lebanon	1,407	809	11	85
Libya	154	55	15	75
Morocco	1,185	651	6	91
Saudi Arabia	156	49	6	91
Syria	439	161	7	83
Tunisia	532	319	13	80
UAE	189	113	11	80

The IAEA has the mandate (Article III) to “encourage and assist research on, and development and practical application of, atomic energy”. The Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture supports this by assisting Member States strengthen their capacity for using nuclear methods to improve technologies for sustainable food security [10]. Among the nuclear techniques that are used in agricultural research [11], at least two are readily applicable to fertigation studies: i) the soil moisture neutron probe, which facilitates the monitoring of soil water status and water-balance evaluation [12], and ii) isotopically-labelled mineral fertilizers, which facilitate the study of their fate in the soil-plant system [13,14].

It is recognized that drip irrigation/fertigation is an effective means of conserving water and increasing fertilizer use efficiency. However, although fertigation was already in wide use in West Asia in the 1990s, insufficient information was available for most crops concerning the most efficacious levels of nutrients and forms of fertilizer. Moreover, in some cases, low fertilizer recoveries, due to the intensive fertilization practised during the last few decades, had created serious agricultural and environmental problems. Treated wastewater is being used in some West Asian countries, however, its impact on the environment requires careful study, especially when used for drip irrigation/fertigation of crops yielding food that is eaten uncooked [15].

An IAEA Regional Technical Co-operation Project (TCP) was implemented during the period 1995 to 1998 on “Water balance and fertigation for crop improvement (RAW/5/002).” Scientists in eight countries participated in the research: Turkey, Cyprus, Syria, Jordan, Lebanon, Iran, Saudi Arabia, and the United Arab Emirates, with the aim of applying nuclear techniques to identify best management practices for increased crop yields in arid and semi-arid zones.

## 2. MATERIALS AND METHODS

The main objectives of the TCP may be summarized as follows:

- to compare the conventional fertilization method with fertigation,
- to evaluate the efficiency of use of N applied with the conventional method as compared to fertigation,
- to evaluate water use efficiency and estimate crop water requirements under conventional N-fertilization and fertigation at three different rates,
- to evaluate potential  $\text{NO}_3^-$ -N pollution with conventional fertilization and fertigation,
- to demonstrate the benefits of the technology to farmers, and assist in its transfer, following evaluation of the collected data.

To achieve the above objectives the following treatments were proposed both for greenhouse and field conditions:

- $N_0$  = zero N application,
- $N_1$  = Half of the locally recommended rate applied by fertigation (50% of  $N_2$ ),
- $N_2$  = The locally recommended rate of N applied by fertigation,
- $N_3$  = Fifty percent more than the locally recommended rate applied by fertigation (150% of  $N_2$ ),
- $N_s$  = N application to the soil at the same rate as treatment  $N_2$ .

In the  $N_s$  treatment, the fertilizer-N was applied according to traditional farming practice. In some cases, the whole amount was applied at planting, in others it was split into two or more applications, with the first applied through the irrigation system and later splits applied to the soil. The overall amount applied in the  $N_2$  treatment through the irrigation system (fertigation) with every irrigation was equivalent to the amount of the soil-N application ( $N_s$  treatment). The amount of N applied with the  $N_s$  treatment was the amount that is recommended to farmers for a particular crop with the conventional method of fertilization, based on soil fertility. The  $N_1$  and  $N_3$  concentrations were equally spaced from  $N_2$ . For example, if  $N_2$  was 100 mg N/L in the irrigation water, then  $N_2$  was recommended to be 50 mg N/L and  $N_3$  to be 150 mg N/L.

A randomized complete block design was recommended of four to eight replicates, each divided into five plots. The plots, depending on the crop, contained one, three or five drip-irrigated rows. Row length depended on the space available, but, in most cases, ranged from 6 to 10 m. The spacing between the rows depended on the crop, but was sufficient to avoid interference from the rows neighbouring the one to which  $^{15}\text{N}$ -labelled fertilizer was applied. In cases of insufficient spacing between the rows, plastic or metal barriers were inserted between the rows at the place of the  $^{15}\text{N}$  application. For three or five rows, the central or the three central rows, respectively, were used for yield and other data collection.

The  $^{15}\text{N}$ -labelled fertilizer was applied as follows:

- For soil application ( $\text{N}_s$ ), the  $^{15}\text{N}$ -labelled fertilizer was applied in the centre of the experimental row at a distance such that it was irrigated by three or five drippers (one or several split applications according to local practice).
- In all fertigated plots, the  $^{15}\text{N}$ -labelled fertilizer was applied through inverted bottles via a dripper or a clip [13]. Where the inverted bottles supplied the  $^{15}\text{N}$ -labeled fertilizer, the irrigation line was without drippers. In this way, all plants were irrigated and fertilized through the system except those fertigated with  $^{15}\text{N}$ -labelled fertilizer. The amount of water and the  $^{15}\text{N}$ -labelled fertilizer applied through the bottles was equivalent to the amount applied through a single dripper.

Phosphorus and K were applied uniformly to all treatments through the irrigation system. The irrigation/fertigation system was composed of two hydraulic injectors with five main lines of plastic tubing in which the five N rates were injected with one to five lateral lines for each crop. The drippers were spaced on the laterals at the distance of planting. One hydraulic injector was used for supplying all treatments with uniform concentrations of P and K. To produce the N levels for the three fertigation treatments, N-fertilizer was injected by the second hydraulic injector in the ratio of 1:2:3 in the irrigation stream for the  $\text{N}_1$ ,  $\text{N}_2$  and  $\text{N}_3$  treatments, respectively [16]. Water requirements and water use efficiency were evaluated using a soil moisture neutron probe and several access tubes (1.5 m in depth) in all treatments. At least two to four replications were observed with one access tube next to the dripper, another on the line between two drippers, and possibly a last one between two laterals. Two tensiometers or tensionics were installed beneath drippers in the  $\text{N}_2$  treatment for irrigation scheduling and assessment of any leaching of nitrate [17].

### 3. RESULTS AND DISCUSSION

#### 3.1. Iran

Tomatoes were grown with urea applied at 0, 150, 300 and 450 mg N/L under drip irrigation [18]. The conventional treatment with surface irrigation received 500 kg N/ha. Drip irrigation saved about 50% of irrigation water (Fig. 1). Yield increased dramatically under drip irrigation, whereas increased rates of N-fertilizer  $\text{N}_1$  and  $\text{N}_2$  had no significant effect on fruit dry matter yields.

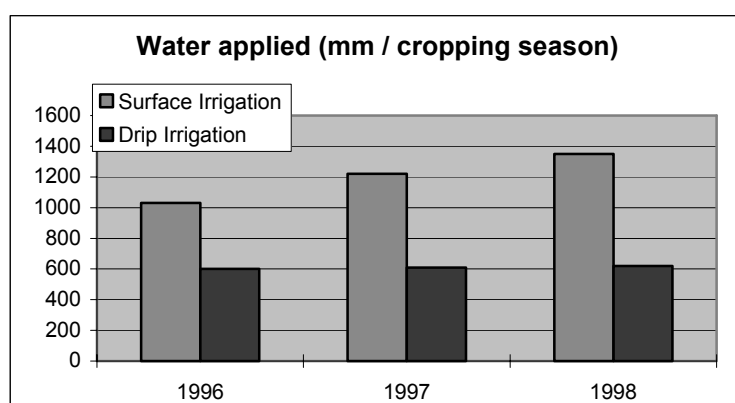


FIG. 1. Amounts of irrigation water applied, tomato, Iran.

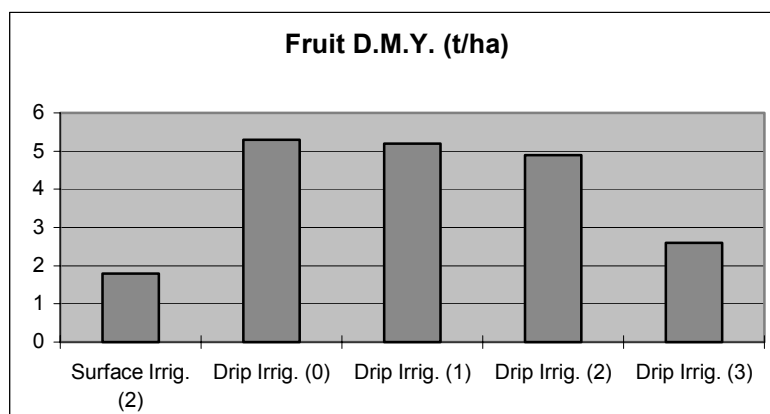


FIG. 2. Tomato fruit dry matter yield vs. treatment, Iran.

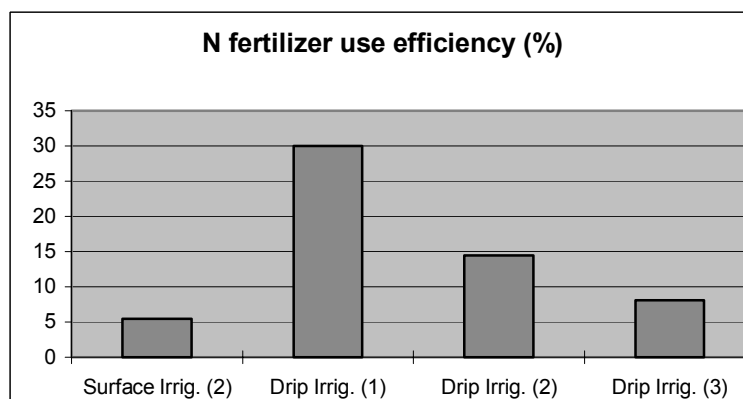


FIG. 3. Fertilizer-N use efficiency vs. treatment, tomato, Iran.

A negative effect on fruit yield was observed in the  $N_3$  treatment (Fig. 2). The fertilizer-N use efficiency (NUE) was very low under surface irrigation (5%), whereas drip fertigation increased the recovery of fertilizer-N to 30% (Fig. 3). In conclusion: drip irrigation significantly increased yields, water use efficiency and fertilizer-N recovery. However, the crop yield response curve is questionable and further studies are underway.

### 3.2. Jordan

#### 3.2.1. Tomato

Tomatoes were grown with ammonium sulphate applied at 0, 50, 100 and 150 mg N/L through fertigation [19,20]. The conventional treatment,  $N_s$ , with 170 kg N/ha, was also drip irrigated. As a consequence, all the treatments received the same amount of water (200 mm of irrigation plus 300 mm of rain). Yield increased significantly with  $N_1$  (Fig. 4). At higher N rates there was no further yield increase. During the 1997–98 season, the N applied to the soil ( $N_s$ ) was significantly less efficient than the corresponding  $N_2$  treatment. The fertilizer-N balance evaluation using  $^{15}N$  recovery for the cropping seasons 1996–97 and 1997–98 are given in Tables II and III, respectively. The N uptake increased significantly with  $N_1$ , but did not increase further at higher rates. It is noteworthy that NUE was highest at  $N_1$  and decreased dramatically with higher N rates. In both cropping seasons, the NUE of the  $N_s$  treatment was significantly lower than with the  $N_2$  treatment. In conclusion, the crop responded positively to the lowest N-fertilizer rate, but no further at higher rates; N-fertilizer applied to the soil was not used efficiently; it was recommended to apply relatively low rates of N through fertigation (e.g. 100 kg N/ha, depending on site characteristics).

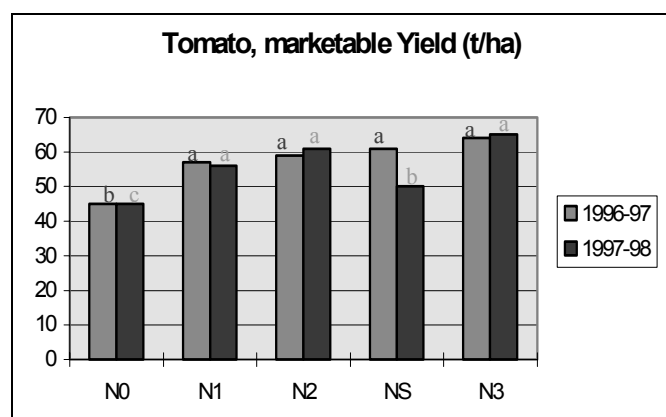


FIG. 4. Tomato yield vs. N treatment, Jordan (statistical comparisons are within-year).

TABLE II. 1996–97, FERTILIZER-N BALANCE, TOMATO, JORDAN

Treatment	N (%)	N uptake (kg/ha)	Ndff (%)	NUE (%)
N <sub>0</sub> (0)	1.5b	97c	—	—
N <sub>1</sub> (84) <sup>a</sup>	2.1a	135b	29a	47a
N <sub>2</sub> (168)	2.1a	135b	21a	17b
N <sub>S</sub> (168)	2.3a	125b	10b	5c
N <sub>3</sub> (252)	2.0a	150a	11b	7c

<sup>a</sup>N applied in kg/ha.

TABLE III. 1997–98, FERTILIZER-N BALANCE, TOMATO, JORDAN

Treatment	N (%)	N uptake (kg/ha)	Ndff (%)	NUE (%)
N <sub>0</sub> (0)	1.7b	140b	—	—
N <sub>1</sub> (64)	2.2a	204a	15a	48a
N <sub>2</sub> (128)	1.9a	201a	11b	17b
N <sub>S</sub> (175)	1.6b	166b	6c	6d
N <sub>3</sub> (192)	1.9a	221a	13b	11c

### 3.2.2. Garlic

Garlic was grown with ammonium sulphate applied at rates of 0, 60, 120, and 180 kg N/ha (1997) and 0, 75, 150, and 225 kg/ha (1998) through fertigation [20]. Two conventional treatments, N<sub>S1</sub> and N<sub>S2</sub>, consisted of surface application of 120 kg N/ha in one dose and two splits, respectively. Weekly water consumption ranged from 10 mm at the beginning of the growing season to 40 mm at mid-season. Yields increased significantly with N<sub>1</sub> and N<sub>2</sub> in 1997, but only with N<sub>1</sub> in 1998 (Fig. 5 and Fig. 6). Treatment N<sub>S2</sub> always gave higher yields than did N<sub>S1</sub>. Fertilizer-N recovery values for 1997 and 1998 are shown in Tables IV and V, respectively. Total N uptake increased with N<sub>1</sub> and N<sub>2</sub>, with mean N-recovery values of

20 to 30%. Rate N<sub>3</sub> was not efficient in terms of yield nor of NUE. The conventional treatments, N<sub>s1</sub> and N<sub>s2</sub>, gave rather low values for total N uptake, as well as for fertilizer-N recovery, which ranged from 5 to 10%. In conclusion, garlic yields increased with N rates especially under fertigation; maximum yields were reached with 80 kg N/ha (fertigation) or 120 kg N/ha (conventional application); fertilizer-N recovery was higher under fertigation; split application is recommended for conventional application.

\

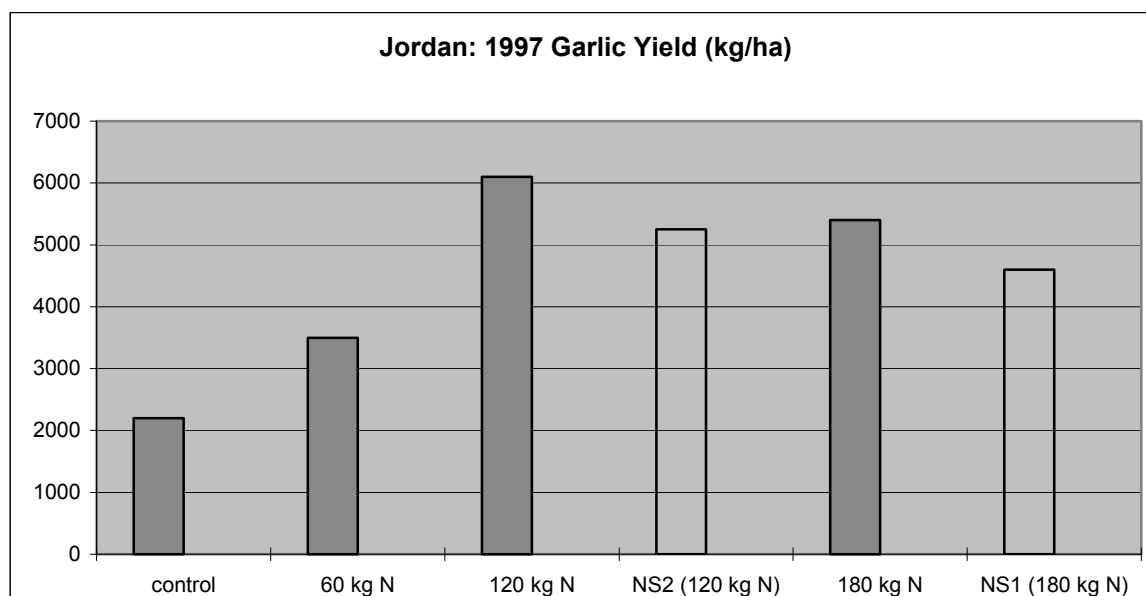


FIG. 5. Garlic yield vs. N treatment, 1997, Jordan.

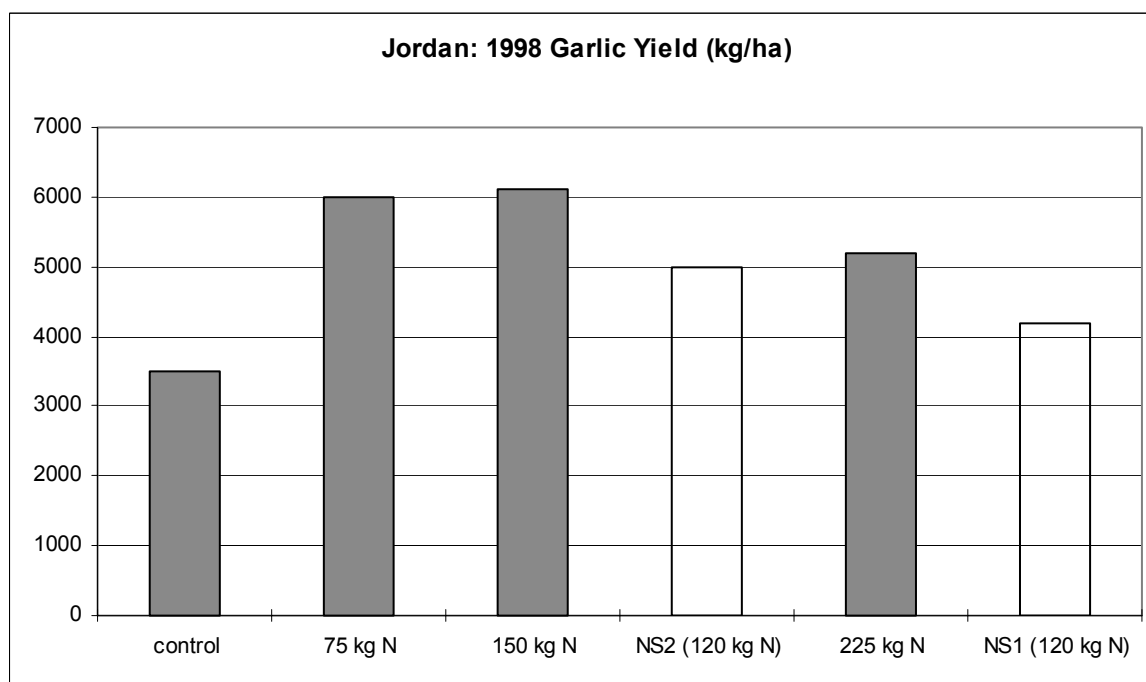


FIG. 6. Garlic yield vs. N treatment, 1998, Jordan.

TABLE IV. 1997, FERTILIZER-N RECOVERY, GARLIC, JORDAN

N rate (kg/ha)	Fruit uptake (kg N/ha)	Shoot uptake (kg N/ha)	Ndff fruit (%)	Ndff shoot (%)	N recovery (%)
60	44	7	21	16	17
120	70	14	29	22	20
180	67	19	41	31	18
NS <sub>2</sub> (120)	55	10	20	20	11
NS <sub>1</sub> (180)	50	5	12	12	4

TABLE V. 1998, FERTILIZER-N RECOVERY, GARLIC, JORDAN

N rate (kg/ha)	Fruit uptake (kg N/ha)	Shoot uptake (kg N/ha)	Ndff fruit (%)	Ndff shoot (%)	N recovery (%)
75	71	11	27	25	29
150	78	14	32	30	19
225	69	14	34	33	13
NS <sub>2</sub> (120)	57	6	23	16	12
NS <sub>1</sub> (120)	46	5	19	19	8

TABLE VI. SOIL RESIDUAL NO<sub>3</sub><sup>-</sup> (mg/kg SOIL), POTATO, LEBANON

Treatment	Soil layer (cm)		
	0–20	20–40	40–60
Initially	11	13	12
End N <sub>0</sub>	29	12	12
End N <sub>1</sub> (240)	47	35	16
End N <sub>2</sub> (360)	96	48	27
End Ncs (360)	32	59	42
End Ncd (360)	36	21	31
End N <sub>3</sub> (480)	122	35	35

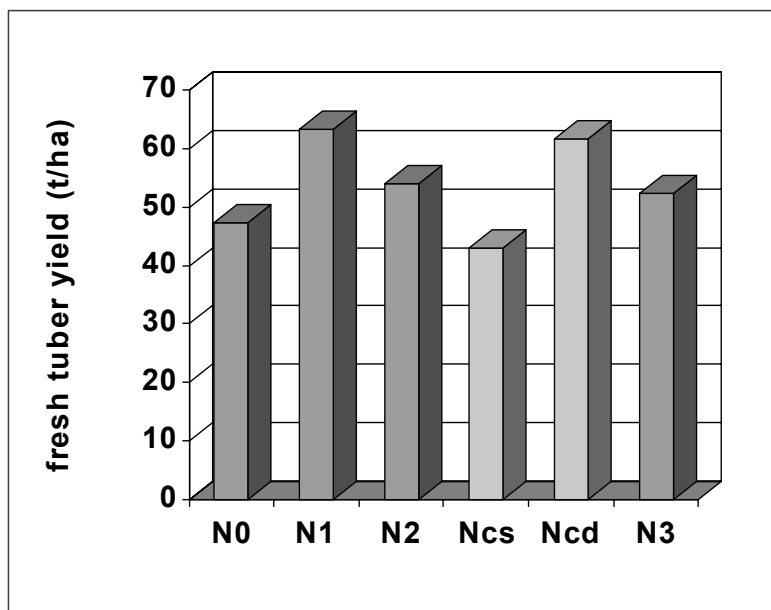


FIG. 7. Spring potato yields (1997), Lebanon.

### 3.3. Lebanon

#### 3.3.1. Potato

Field potatoes were grown with rates of urea ranging from 0 to 480 kg N/ha (1997) and 0 to 360 kg N/ha (1998) [21]. There were six treatments ( $N_{0-3}$ ,  $N_{cd}$ , and  $N_{cs}$ ) and four replicates. The conventional fertilizer-N applications were irrigated either by drip ( $N_{cd}$ ) or sprinkler ( $N_{cs}$ ). Drip irrigation saved 42% of irrigation water: 500 mm compared with 860 mm through sprinkler irrigation. Yield responded positively to N under drip irrigation at the low rate  $N_1$  (Fig. 7). Conventional fertilization was not efficient under sprinkler irrigation; it was improved by drip irrigation. The N recovery was around 25% (Fig. 8). Owing to this low, a major part of the N-fertilizer applied was left in the soil after harvest (Table VI), and was thus susceptible to leaching during the wet season.

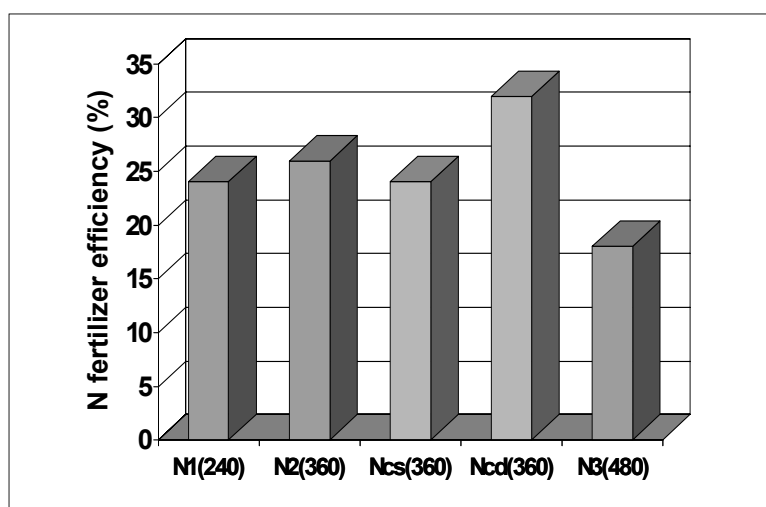


FIG. 8. Fertilizer recovery, (1997), potato, Lebanon.



### 3.3.2. *Cucumber*

Cucumber was grown under greenhouse conditions with two modes of irrigation (every 2 or 3 days) and two modes of fertigation (continuous or discontinuous: every other irrigation). Basic rates of fertilizers were 135 mg N/L as ammonium sulphate, 40 mg P/L as phosphoric acid and 200 mg K/L as potassium sulphate [22, 23]. It was shown that continuous fertigation, applied every second day, gave yields significantly higher than all other treatments (Fig. 9). In terms of NUE, treatments T<sub>2s</sub> were significantly better than treatments T<sub>3s</sub>; otherwise there was no significant difference between sub-treatments C and D (Fig. 10). Postponing irrigation and fertigation induced an accumulation of nitrate in the upper soil layers (Table VII). This accumulation was associated with increased soil electrical conductivity (Table VIII).

TABLE VII. RESIDUAL NITRATE-N (ppm)  
AT HARVEST, CUCUMBER, LEBANON

Treatment	Soil layer (cm)		
	0–20	20–40	40–60
Initially	61a	54	33
End T <sub>2</sub> C	62a	51	18
End T <sub>2</sub> D	82a	51	23
End T <sub>3</sub> C	126b	62	19
End T <sub>3</sub> D	111b	68	18

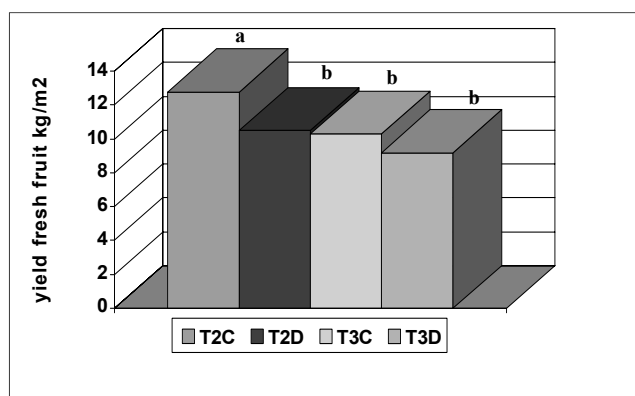


FIG. 9. Cucumber yield vs. T2-3/C-D, Lebanon.

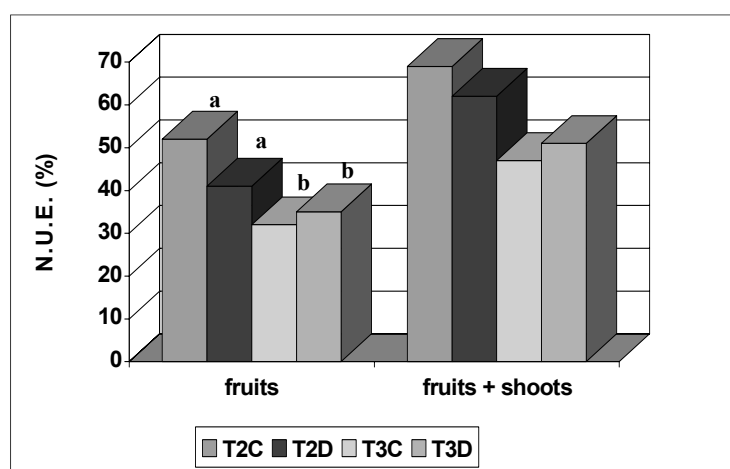


FIG. 10. N use efficiency, cucumber, Lebanon.

TABLE VIII. SOIL ELECTRICAL CONDUCTIVITY (dS/m), LEBANON

Treatment	Soil layer (cm)		
	0–20	20–40	40–60
Initially	2.2a	1.9	2.1
End T <sub>2</sub> C	2.4a	1.8	1.1
End T <sub>2</sub> D	3.9b	2.2	1.3
End T <sub>3</sub> C	3.9b	2.7	1.4
End T <sub>3</sub> D	3.7b	2.5	1.6

### 3.4. Saudi Arabia

Cucumbers were grown under greenhouse conditions on a typical coarse sandy soil (6–9% field capacity). Five treatments (0–300 kg N/ha, treatment N<sub>soil</sub> being the conventional rate) were imposed with six replicates [24]. Yields responded positively to increased rates of N-fertilizer (Fig. 11), but did not reach a plateau; 200 kg N/ha is generally regarded as optimal and is locally recommended. The treatment N<sub>soil</sub> was less effective than the equivalent rate of N-fertilizer used through drip irrigation. On the other hand, cucumber mineral contents (N, P and K) increased significantly with increasing N-fertilizer rates (Fig. 12). The N-recovery data are still pending. Nevertheless, there is much potential for adoption of fertigation practices for increasing production of greenhouse crops.

### 3.5. Syria

Field experiments were initiated with cotton with the following objectives [25]: to assess fertilizer-N use efficiency under conventional (three split soil-surface applications) and fertigation practices, to evaluate N requirements of cotton under fertigation, and to study the comparative water use efficiency of cotton grown under these conditions. Irrigation requirements, over the period 1995–98, were about half under drip irrigation compared with furrow irrigation (Fig. 13).

Yields increased with N-fertilizer rates, reaching a plateau around 120 kg N/ha under fertigation. The conventional treatment, N<sub>cs</sub>, consistently gave significantly lower yields than the corresponding N<sub>3</sub> treatment; often it was no better than N<sub>0</sub> (Fig. 14). During the period 1995–98, the last two years were characterized by higher NUE values under all treatments (Fig. 15); one possible explanation is better timing of N application and the final irrigation. The results showed the superiority of fertigation over surface-irrigation. Nitrate monitoring of the soil solution at increasing depths, using sets of tensionics, indicated that leaching of fertilizer-N may be one of the reasons for the ineffectiveness of surface irrigation (Fig. 16).

### 3.6. Turkey

Eight experiments were conducted under greenhouse conditions with tomato, pepper, cucumber, melon, and eggplant on a terrarosa soil near Antalya [26]. Data obtained with tomato (three trials) are reported here. Fertilizer rates ranged from 0 to 150 mg N/L with a conventional treatment corresponding to the N<sub>2</sub> rate, which supplied 300 kg N/ha as ammonium sulphate: one-third before planting, and two-thirds during particular growth stages. Yields responded positively to N<sub>1</sub>, the first rate of fertilizer, but not beyond it (Fig. 17). In two out of three experiments, the conventional treatment N<sub>c</sub>(300) gave significantly lower yields than N<sub>2</sub>(300) in which the same amount of N was applied through drip irrigation.

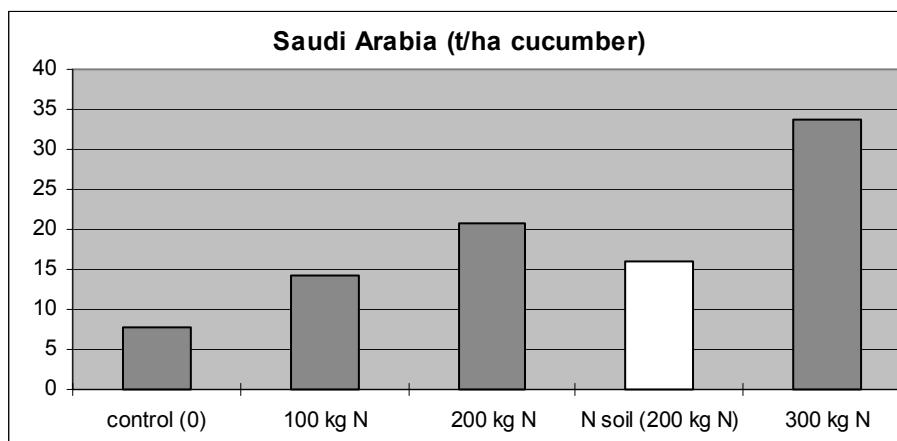


FIG. 11. Cucumber yield vs. N-application rate, Saudi Arabia.

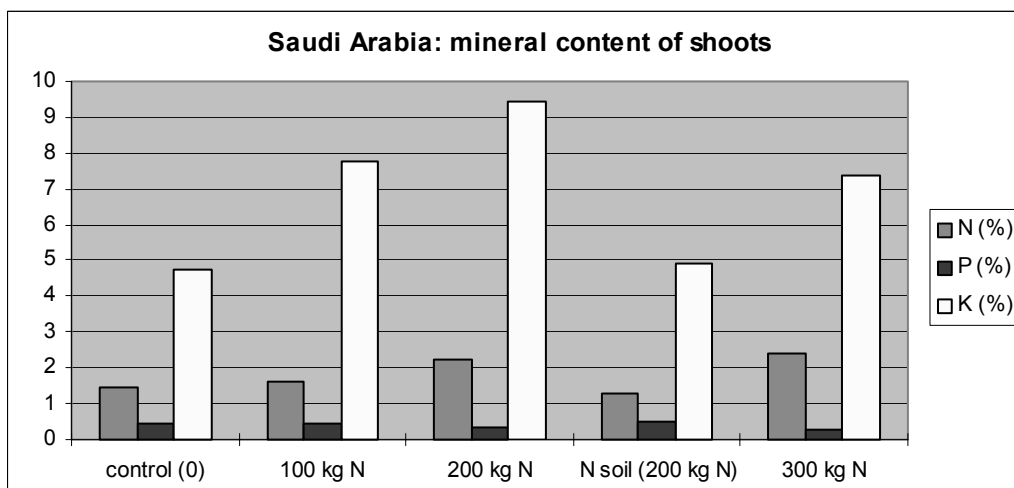


FIG. 12. Mineral content of cucumber shoots vs. N-application rate, Saudi Arabia.

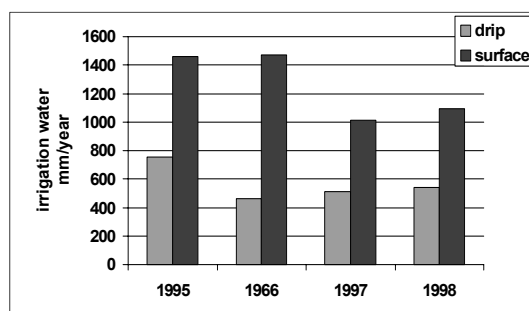


FIG. 13. Irrigation requirements of cotton, Syria.

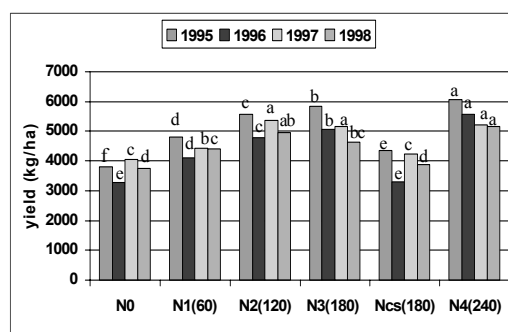


FIG. 14. Seed cotton yield vs. treatment, Syria (statistical comparisons are within-year).

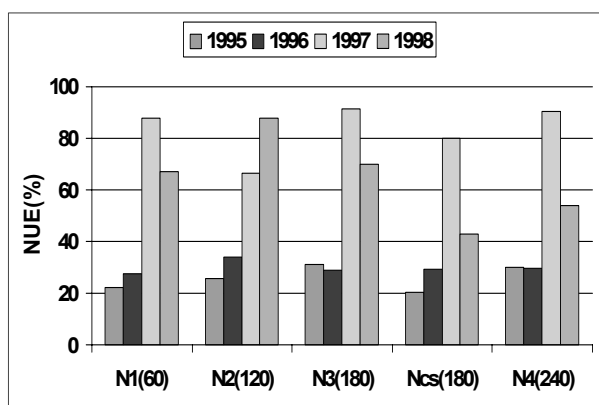


FIG. 15. Fertilizer-N use efficiency, cotton, Syria.

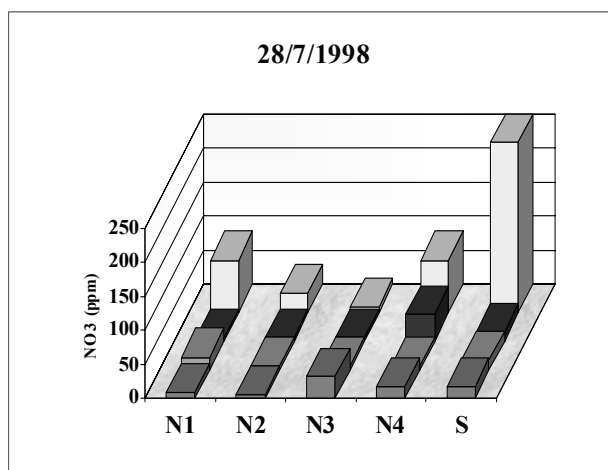


FIG. 16. Nitrate conc. in the soil solution at 25-cm increments in depth vs. treatments, cotton, Syria [25].

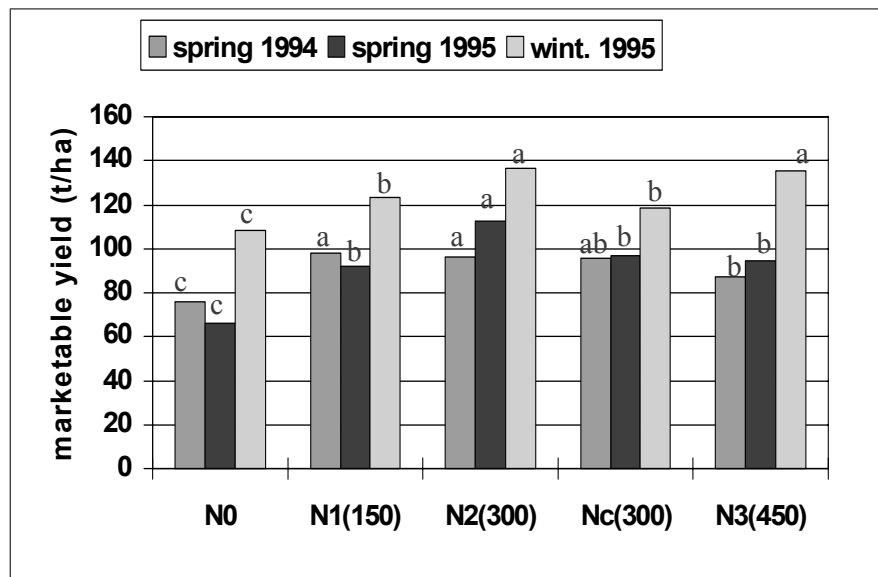


FIG. 17. Tomato yield vs. N, 1994–95, Turkey (statistical comparisons are within-year).

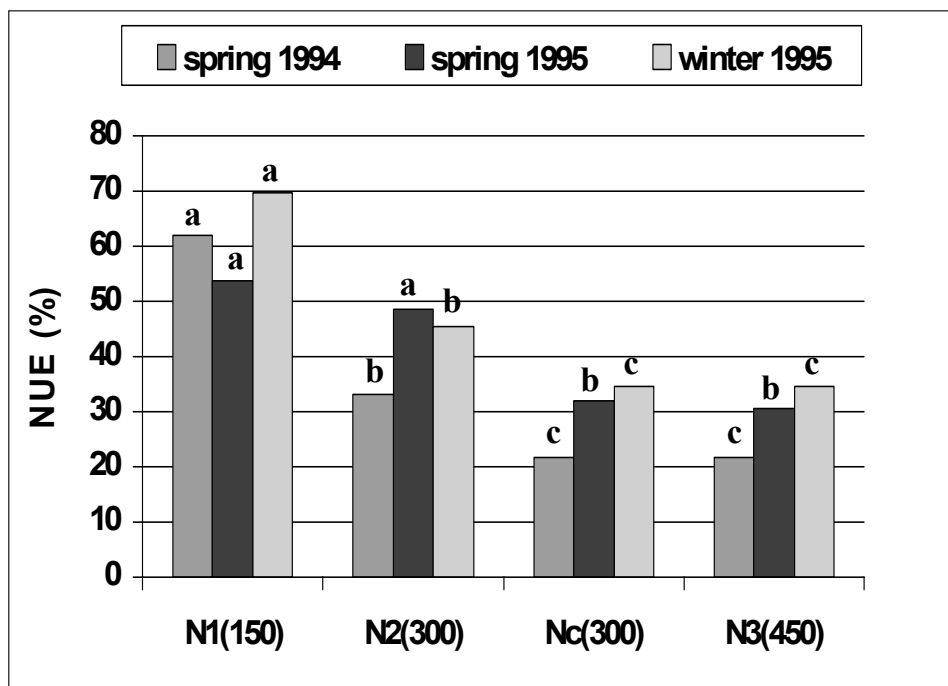


FIG. 18. N recovery, tomato, Turkey (statistical comparisons are within-year).

TABLE IX. ADVANTAGES OF FERTIGATION, TURKEY

Crop	Increase (%)		
	Yields <sup>a</sup>	NUE <sup>b</sup>	WUE <sup>b</sup>
Tomato	50	55	10
Pepper	50	55	10
Melon	45	120	80
Cucumber	65	80	10
Eggplant	50	150	20

<sup>a</sup>N<sub>2</sub> vs. N<sub>0</sub>. <sup>b</sup>N<sub>2</sub> vs. N<sub>c</sub>.

However, the difference in yield was very small owing to the high level of soil fertility and good water-holding capacity. At rate N<sub>1</sub> the N recovery was rather high (50–70%), decreasing at higher N rates (Fig. 18). The recovery of the conventionally applied fertilizer-N was significantly less than with the same amount of N applied through fertigation. A synthesis of the advantages of fertigation is shown in Table IX. Rate N<sub>2</sub> was superior compared with N<sub>0</sub> and N<sub>c</sub> in terms of yields, and efficiencies of use of N and water for the five crops under investigation; consistently, yields and NUE were substantially increased.

### 3.7. United Arab Emirates

Cucumber was grown in the field under protected conditions on a saline (~4 dS/m) sandy loam low in organic matter content. Four rates of N (0, 200, 400, and 600 kg N/ha) were arranged in a CRBD with eight replicates [27]. The plots received 300 and 450 kg/ha of P and K, respectively, as well as 10 t/ha of organic matter. Yields responded positively to the first rate of N-fertilizer, but not beyond (Fig. 19). Low plant density (8,130 units/ha) may partially explain the relatively low yields. Total N uptake (49.8 kg N) was low despite high inputs: 200 kg N/ha plus 10 t organic matter (Table X). The Ndff values reached 30%; at the N<sub>2</sub> rate (200 kg N/ha), which represents a fertilizer-N recovery of only 7%. Further work is in progress to improve crop conditions.

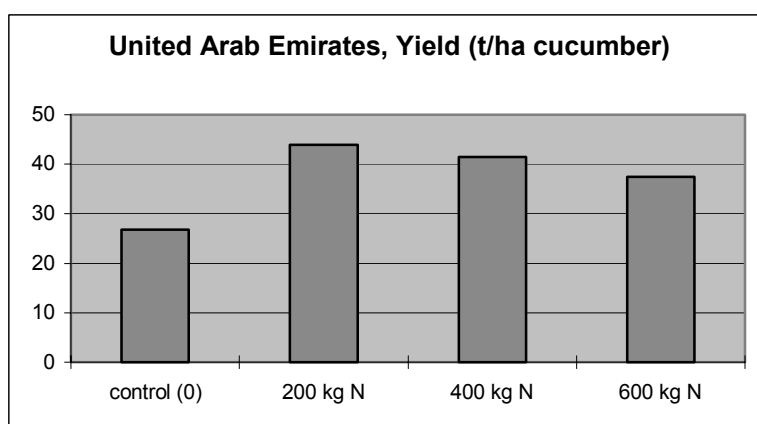


FIG. 19. Cucumber yield vs. N-rate, United Arab Emirates.

TABLE X. N-YIELD OF CUCUMBER PLANT PARTS AT 200 kg/ha,  
UNITED ARAB EMIRATES

Plant part	DMY (t/ha)	Total N (%)	N yield (kg/ha)	Ndff (%)	Fert. N yield (kg N/ha)
Fruits	1.32	2.76	36.6	29	10.6
Shoots	0.61	2.09	13.0	31	4.1
Roots	0.01	1.58	0.24	31	0.1
Total	1.94		49.8	30	14.8

#### 4. CONCLUSIONS AND RECOMMENDATIONS

Over 4 years (1995–98), experiments undertaken in eight countries within Europe and West Asia have shown that drip irrigation and fertigation are promising techniques for the region. “On-station” trials were conducted according to the guidelines for the Regional Technical Co-operation Project RAW/5/002 on “Water balance and fertigation for crop improvement.” Experiments benefited from nuclear techniques (soil moisture neutron probe,  $^{15}\text{N}$ -labelled fertilizers) and from the support of the FAO/IAEA programme in fertigation studies in the Mediterranean region. The main objective was to quantify possible improvements in efficiency of use of water and of fertilizer-N through drip irrigation and fertigation. It was shown that:

- 30 to 50% of irrigation water can be saved under drip irrigation;
- fertilizer-N application (as well as P and K inputs in most of the cases) is a necessity for maintaining the productivity of the irrigated crops investigated. On control treatment ( $\text{N}_0$ ) yields were 0 to 80% lower than  $\text{N}_{(1-3)}$ , depending on the original soil-fertility level;
- the conventional application of N-fertilizer to the soil itself (pre-planting plus split applications later) was not effective whatever the mode of irrigation employed, even if drip irrigation itself gave higher yields compared with conventional techniques;
- under fertigation at increasing rates of N-fertilizer, the optimal crop yield was often reached at rate  $\text{N}_1$  instead of rate  $\text{N}_2$  which corresponded to the “locally recommended rate”; therefore, fertigation could promote N-fertilizer savings of 50% of the recommended inputs under conventional irrigation.

#### ACKNOWLEDGEMENTS

The IAEA thanks the national institutions and staff who contributed to this project: their names are listed with Refs. [18] to [27]. They have all been involved in RAW/5/002 Project, and their commitment to implementing the studies described is noteworthy. Support was also provided by the IAEA Technical Co-operation Department, in particular the contributions of Mr. S. Chaudhri, Section Head of the West Asia Region, and Mr. A. Habjouqa, Project Officer, are gratefully acknowledged.

#### REFERENCES

- [1] MOORE, J.W., Balancing the Needs of Water Use, Springer-Verlag, New York (1988). ISBN 0-387-96709-5
- [2] KEMP, P., New war of words over scarce water, Middle East Economic Digest **49** (1996) 2–7.
- [3] PIMENTEL, D., et al., Water resources: agriculture, the environment, and society, BioScience **47** (1997) 97–106.
- [4] TUIJL, W., Improving Water Use in Agriculture: Experience in the Middle East and North Africa, World Bank, Washington, DC (1993).

- [5] HILLEL, D., *Rivers of Eden, The Struggle for Water and the Quest for Peace in the Middle East*, Oxford University Press, Oxford (1994). ISBN 0 19 508068 8
- [6] PITTS, D.J., CLARK, G.A., Comparison of drip irrigation for tomato production in South West of Florida, *Applied Eng. Agric.* **7** (1991) 177–184.
- [7] POLAK, P., et al., A low cost drip irrigation system for small farmers in developing countries, *Water Resources J. ESCAP* **195** (1997) 26–31.
- [8] NARAYANAMOORTHY, A., Beneficial impact of drip irrigation: a study based on Western India, *Water Resources J. ESCAP* **195** (1997) 17–25.
- [9] Fertilization/Chemigation (Proc. Expert Consultation, Cairo, 1991) AGL/MISC/19/91, FAO Rome (1991).
- [10] INTERNATIONAL ATOMIC ENERGY AGENCY, Annual Report, Highlights 97 Food and Agriculture, IAEA/PI/A22E, IAEA, Vienna (1997).
- [11] INTERNATIONAL ATOMIC ENERGY AGENCY Use of Nuclear Techniques in Studies of Soil-Plant Relationships, IAEA Training Course Series No. 2, (HARDARSON, G., Ed.), IAEA, Vienna (1990).
- [12] KIRDA, C., “Use of neutron water and gamma density gauges in soil water studies”, Use of Nuclear Techniques in Studies of Soil-Plant Relationships, IAEA Training Course Series No. 2 (HARDARSON, G., Ed.), IAEA, Vienna (1990) 183–223
- [13] PAPADOPOULOS, I., “Use of labelled fertilizers in fertigation research IAEA-SM-334/18,” Nuclear Techniques in Soil-Plant Studies for Sustainable Agriculture and Environmental Preservation, IAEA, Vienna (1995) 399–410.
- [14] MOUTONNET, P., et al., “Assessment of inorganic N in soil solution using Tensionics, IAEA-SM-334/48”, Techniques in Soil-Plant Studies for Sustainable Agriculture and Environmental Preservation, IAEA, Vienna (1995) 411–418.
- [15] SHATANAWI, M., FAYYAD, M., Effect of Khirbet As-Samra treated effluent on the quality of irrigation water in the central Jordan Valley, *Wat. Res.* **30** (1996) 2915–2920.
- [16] PAPADOPOULOS, I., A fertigation system for experimental purposes, *Plant Soil* **102** (1987) 141–143.
- [17] MOUTONNET, P., FARDEAU, J.C.L., Inorganic Nitrogen in soil solution collected with *tensionics* samplers, *Soil Sci. Soc. Am. J.* **61** (1997) 822–825.
- [18] HOBBI, M.S., SAGEB, N., MOUSAVI SHALMANI, A., KHORASANI, A., ABBASALIAN, H., Field Evaluation of Urea Fertilizer and Water Use Efficiency by Tomato Under Trickle Irrigation (Progress Report RAW/5/002, 31 October to 4 November, 1998, Karaj, Iran), IAEA, Vienna (1998).
- [19] AL-ZURAIQI, S., AL-QAWASMI, W., MOHAMMAD, M.J.R., AL-DEEK, I., Management of Nitrogen Fertigation on Tomato Crops With the Use of <sup>15</sup>N Technology (Progress Report RAW/5/002, 31 October to 4 November, 1998, Karaj, Iran), IAEA, Vienna (1998).
- [20] MOHAMAD, M.J.R., OMARI, M., ZURAIQI, S., QAWASMI, W., Nitrogen and Water Utilization of Trickle-Fertigated Garlic Using the Neutron Gauge and <sup>15</sup>N Technology (Progress Report RAW/5/002, 31 October to 4 November, 1998, Karaj, Iran), IAEA, Vienna (1998).
- [21] DARWISH, T., ATTALAH, T., HAJHASAN, S., CHRANEK, A., Fertigation of spring potatoes utilising <sup>15</sup>N in Central Beqaa, Lebanon: comparative water and N-fertilizer utilisation in fertigation vs. soil application under drip and macro sprinkler systems (Progress Report RAW/5/002, 31 October to 4 November, 1998, Karaj, Iran), IAEA, Vienna (1998).
- [22] ATTALAH, T., DARWISH, T., EL-MOUJABBER, M., Modality of Fertigation of Protected Cucumber and Nitrogen Use Efficiency Under Field Conditions (Progress Report RAW/5/002, 31 October to 4 November, 1998, Karaj, Iran), IAEA, Vienna (1998).
- [23] EL-MOUJABBER, M., ATTALAH, T., DARWISH, T., Crop Modelling and Water Use Efficiency of Protected Cucumber (Progress Report RAW/5/002, 31 October to 4 November, 1998, Karaj, Iran), IAEA, Vienna (1998).
- [24] AL-WABEL, M.I., AL-JALOOD, A.A., HUSSAIN, G., KARIMULLA, S., Study For Water Balance And Fertigation Crop Improvement (Progress Report RAW/5/002, 31 October to 4 November, 1998, Karaj, Iran), IAEA, Vienna (1998).



- [25] JANAT, M., SOMI, G., Comparative Study of Nitrogen Fertilizer Use Efficiency of Cotton Grown Under Conventional and Fertigation Practices Using  $^{15}\text{N}$  Methodology (Progress Report RAW/5/002, 31 October to 4 November, 1998, Karaj, Iran), IAEA, Vienna (1998).
- [26] HALITLIGIL, M.B., AKIN, A.I., KISLA, H., OZTURK, A., DEVIREN, A., Yields, Nitrogen Uptakes and Nitrogen Use Efficiencies of Tomato, Pepper, Cucumber, Melon and Eggplant as Affected By N-Rates Applied With Drip Irrigation Under Greenhouse Conditions (Progress Report RAW/5/002, 31 October to 4 November, 1998, Karaj, Iran), IAEA, Vienna (1998).
- [27] AL-MEHRAZI, M.S., SATTAR, H., ABU-ALKIBASH, H., Cucumber Nitrogen Need Under Protected Cultivation Using  $^{15}\text{N}$ -Labelled Urea (Progress Report RAW/5/002, 31 October to 4 November, 1998, Karaj, Iran), IAEA, Vienna (1998).

# **PLANT TOLERANCE TO ENVIRONMENTAL STRESS**

(Session 4)



## Keynote Address

### GENETIC DIVERSITY OF PLANTS AND ITS EXPLOITATION FOR STRESS ENVIRONMENTS

S.H.M. NAQVI, R. BILAL

Radioisotope Application Division,

Pakistan Institute of Nuclear Science and Technology (PINSTECH),

Islamabad, Pakistan

Water deficiency and elevated temperatures may be the most important of numerous environment stresses that tend to constrain plant growth and survival. We are addressing moisture stress and its ramifications on the macro and micro levels, and are examining means of increasing agricultural productivity in arid and semi-arid environments.

Although water covers more of the surface of the globe than does land, it is mainly saline. Of all surface waters 97% lies in the oceans, 2% is held in ice caps and only 1% is fresh, originating from precipitation and snowmelt and present in lakes, rivers and biological systems [1]. Of the 1% fresh water, a fraction is harnessed for agricultural, industrial and domestic purposes, with agriculture consuming 70%. Fresh water is a scarce commodity that is distributed unevenly geographically and seasonally, leaving vast areas arid and semi-arid. The worst affected are, perhaps, areas located in the northern and southern sub-tropics. In addition to natural factors, exacerbating forces of human origin are affecting large areas, with global implications.

Plants still provide the most economical and convenient means of harnessing solar energy. Over millennia they have evolved and adapted to varied environmental conditions; they grow on mountains, in the plains, in deserts, submerged in water, and even in the sea. From the photosynthetic capture of photons to the storage of chemical energy, the reactions take place with varied efficiency according to the species and the prevailing environmental conditions. Similarly, in terms of uptake and utilization of water and nutrients, plants possess vast variability, most of which is still grossly under-utilized.

Saline groundwater is present in a number of arid countries. This water could be utilized to help specific plant species, not only to survive but to thrive to produce biomass that could be used as food, forage, fuel, manure, or as industrial feedstock (biosaline agriculture). There is no doubt that halophytes and other salt-tolerant varieties of some species could be grown using saline water [2]. However, the extent of sustainability would depend on controlling salt build-up in the surface of the irrigated soil and by having estimates of the extent of the groundwater reservoir, its dynamics, and qualitative and quantitative changes over time. Nuclear techniques offer a means of obtaining useful, rather critical, information on these issues.

The IAEA has initiated a Model Project to examine the feasibility and extent of sustainability of bio-saline agriculture in eight countries in North Africa, West Asia and South Asia. The project has four main activities/thrusts:

- To introduce salt-tolerant plant species on a demonstration site of at least 10 ha and to select those showing comparative advantage under the prevailing socio-economic conditions.
- To achieve good irrigation management with the help of the neutron moisture gauge (NMG) and supplementary techniques to ensure that salt does not accumulate in the soil surface.
- To regularly monitor (by chemical and isotopic means) the groundwater of the area to estimate quality, quantity, and sources of recharge to ascertain the extent of sustainability of the agricultural activity.
- To pass the technology on to the end-user.

Locally available and exotic salt-tolerant perennial and annual plant species, most of them nitrogen fixing, have been introduced on 10-ha sites in arid wastelands of Morocco, Tunisia, Egypt, Syria, Iran, and Pakistan. Some species of trees, bushes, and grasses known to be salt tolerant, *Acacia*, *Prosopis*, *Casuarina*, *Tamarix*, *Eucalyptus*, *Olea*, pistachio, date palm, *Atriplex*, *Kochia*, *Hordeum*, *Leptochloa*, *Sporobolus*, *Sesbania*, *Brassica*, cactus, and some others, have been successfully grown using groundwater of medium to high salinity. The saline irrigation is being managed using the NMG and regular analyses of the soil. The groundwater(s) in a radius of a several kilometers around each site is monitored for chemical and isotopic (mainly isotopes of H, O, and C) characteristics to obtain information on the quality, quantity, and possible sources of its recharge.

The project aimed at utilizing saline water to help at least some of the selected species not merely to survive but to thrive. It also aimed at using these plants as the initiator and sustainer of processes that ultimately improve the environment. In at least one country, the participating scientists have clearly shown beneficial effects of plant growth on the semi-arid environment in general and on the soil in particular; the surface salinity has decreased, soil structure has improved, and fertility increased. Experience gained on the successful cultivation of some of the introduced species has already been passed on to end users for economic benefit in two countries [3].

With growing pressure on limited water resources, the cultivation of plant species that are tolerant of drought and saline conditions on one hand and the adoption of moisture-conserving techniques on the other, will be critically important for the improvement of degraded soils and for their return to agricultural productivity. Non-destructive isotopic techniques, particularly those based on stable isotopes, need to be developed for convenient selection for tolerance of drought and salinity, for superior water-use efficiency (i.e. better utilizers of solar energy), and for superior nitrogen-fixing capability.

Results indicate that existing genetic variability in plants can be fruitfully exploited not only to grow them in stress environments, but for amelioration of those environments. However, developments in nuclear and molecular techniques are needed to tailor plants for specific situations [4].

## REFERENCES

- [1] FOOD AND AGRICULTURE ORGANIZATION, State of Food and Agriculture, FAO, Rome (1993).
- [2] BOARD OF SCIENCE AND TECHNOLOGY FOR INTERNATIONAL DEVELOPMENT, Saline Agriculture, Salt Tolerant Plants for Developing Countries, Report of a Panel for BOSTID, National Academy Press, Washington, DC (1990).
- [3] QURESHI, R.H., BARRET-LENNARD, E.G., (Eds.), Saline Agriculture for Irrigated Land in Pakistan, ACIAR, Bruce, ACT (1998).
- [4] SHANNON, M.C., "Adaptation of plants to salinity," Advance in Agronomy Volume 60 Academic Press, London (1997). ISBN 0120007606

## ARE PLANTS IN THE CHINESE TAKLAMAKAN DESERT WATER LIMITED? A STABLE-ISOTOPE APPROACH

S.K. ARNDT, A. FOETZKI\*, F.M. THOMAS\*, M. POPP

Institute of Ecology and Conservation Biology,  
University of Vienna,  
Vienna, Austria

### Abstract

A stable isotope approach was used to investigate water-use efficiency and water availability in three species of the natural vegetation surrounding Qira oasis at the southern fringe of the Taklamakan desert in China. Leaf samples of the perennial species *Alhagi sparsifolia*, *Tamarix ramosissima*, and *Populus diversifolia* were collected every 4 weeks during the vegetative period between April and October 1999. Leaf-tissue values for  $\delta^{13}\text{C}$  were most positive at the beginning of the vegetative period and became more negative during the summer months, the time of highest temperatures and greatest water-vapour deficits. More-negative  $\delta^{13}\text{C}$  values in  $\text{C}_3$  plants are indicative of lower water-use efficiency (WUE) and, consequently, in this study, of a non-restricted water supply. From the available stable-isotope data, it can be concluded that all plants were adequately supplied with moisture, and did not suffer drought stress throughout the year despite the extremely arid climate of the Taklamakan desert. Therefore, all species were phreatophytes and had constant contact with groundwater sources.

### 1. INTRODUCTION

The Taklamakan desert in western China is climatically the most extreme desert in Central Asia. Cold winters (January daily mean,  $-8^\circ\text{C}$ ), warm summers (July daily mean,  $26^\circ\text{C}$ ), large temperature fluctuations, frequent sand storms, an annual precipitation of 35 mm, and high water-vapour deficits throughout the year [1], represent a harsh environment for plants. However, at the southern rim of the Taklamakan, along the ancient silk route, thriving plant life can be observed. Snow and glacier melt in the nearby Kunlun mountains frequently cause floods in the summer months and enable an irrigation-oasis agriculture [2]. The indigenous vegetation surrounding the oases is important because it prevents sand-dune movement and provides fuel and timber for local people and fodder for livestock. Due to increasing population pressure, the natural vegetation has been overused at many oases resulting in desertification and devastation of arable land.

To make recommendations for sustainable use of desert species, the ecology of indigenous vegetation has to be understood in greater detail. Observations of local researchers and farmers led to the conclusion that summer floods might be beneficial or a prerequisite for plant survival in the oasis foreland. To investigate plant adaptation to the environment, and tolerance mechanisms to drought as an environmental stress factor, a stable-isotope approach was used to study water-use efficiency and water availability in three species indigenous to Qira oasis at the southern fringe of the Taklamakan desert.

### 2. MATERIAL AND METHODS

Field sites of the legume *Alhagi sparsifolia*, the salt-tolerant salt cedar *Tamarix ramosissima*, and the poplar tree *Populus diversifolia* were established at Qira oasis (N  $37^\circ00.966'$  E  $80^\circ43.767'$ ). All plant species are dominant members of the indigenous vegetation surrounding the oases in southern Xinjiang; plots were placed in areas where each of the species is dominant. Leaf material for C isotope determination was collected from early spring (April) to autumn (October) 1999 every 4 weeks. In a complementary study in August 2000, leaf material was collected from a broader range of species to compare C-isotope signatures:

---

\*Present address: Department Ecology and Ecosystem Research, AvH Institute for Plant Sciences, University of Göttingen, Untere Karspüle 2, D-37073 Göttingen, Germany.

- C<sub>3</sub>-trees, *Eleagnus angustifolia*, *Populus alba*, *Populus diversifolia*;
- C<sub>3</sub>-shrubs, *Tamarix ramosissima*, *Karelinia caspica*, *Scorzonera divaricata*, *Zygophyllum* sp.;
- C<sub>3</sub> grasses, *Phragmites communis*;
- C<sub>4</sub> plants, *Calligonum caput-medusae*, *Halogeton arachnoideus*, *Salsola ruthenica*, *Tribolus terrestris*.

A stable-isotope approach was used to investigate the long-term water relations of the plants. The natural abundance of <sup>13</sup>C (δ<sup>13</sup>C value) in leaf tissue of most C<sub>3</sub> species is related to whole-plant water-use efficiency (WUE) [3]. The reason for this is that a higher discrimination against <sup>13</sup>C relative to <sup>12</sup>C during photosynthesis and respiration occurs in water-sufficient plants compared to drought-stressed counterparts. Thus, increased (more positive) values for δ<sup>13</sup>C in leaves suggest that higher WUE resulted primarily from stomatal control of water loss to maintain plant moisture status in times of water restriction. Therefore, δ<sup>13</sup>C values of plants can be used as an integrated long-term measure of water deficit.

Pulverized bulk leaf samples were analysed by continuous-flow gas isotope ratio mass spectrometry (IRMS, Delta<sup>PLUS</sup>, Finnigan MAT, Bremen, Germany). A detailed description of the IRMS set-up is given in Ref. [4].

The <sup>13</sup>C natural abundance (‰) was calculated as follows:

$$\delta^{13}\text{C} = \frac{R_{\text{sample}}}{R_{\text{standard}} - 1} \times 1000 \quad \delta^{13}\text{C} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$$

where

R is the ratio of mass 45:mass 44 (CO<sub>2</sub>),  
and R<sub>standard</sub> is Vienna Pee Dee Belemnite (VPDB) (‰).

### 3. RESULTS AND DISCUSSION

At the first harvest in April 1999, the δ<sup>13</sup>C values of the leaves of all three species were significantly more positive than those for the remaining vegetative period (Fig. 1). It is likely that the bulk of C in the young leaves was derived from reserve carbohydrate pools in the roots that had a more-positive <sup>13</sup>C signature due to metabolic processing. Furthermore, it is also possible that the developing leaves were more susceptible to water loss and that reduced transpiration led to increased WUE and thus to more-positive δ<sup>13</sup>C values.

During the subsequent months (May–October, 1999), the δ<sup>13</sup>C values of leaf tissue decreased to more-negative values. Even in July and August, the months of peak insolation and highest temperatures, and thus the highest water-vapour deficits, no increases in δ<sup>13</sup>C values were observed. Although there were species differences in the absolute values, overall, the <sup>13</sup>C signatures were very negative compared to other species of the extremely arid zone. Published values of δ<sup>13</sup>C in arid-zone plants range from approximately –20 to –26‰ [5]. The mean <sup>13</sup>C value of leaves of the three C<sub>3</sub> species in August 1999 was –26.9‰. The complementary study in August 2000 revealed similar δ<sup>13</sup>C values for perennial C<sub>3</sub> plants (Table I) although the overall community average was slightly more positive (–26.4‰).

Taken together, the δ<sup>13</sup>C data indicate low WUE in the investigated plant species. On the other hand, the constantly negative leaf δ<sup>13</sup>C values might reflect high soil water availability in the oasis forelands. It has been shown in other studies in arid conditions that stomatal restriction and WUE both increase as soil water availability decreases. Leaves of deciduous trees had more-positive δ<sup>13</sup>C values at xeric sites than at mesic sites in a temperate forest in Tennessee [6], and δ<sup>13</sup>C values in all species increased along a soil-moisture gradient in the Sonoran desert [7].

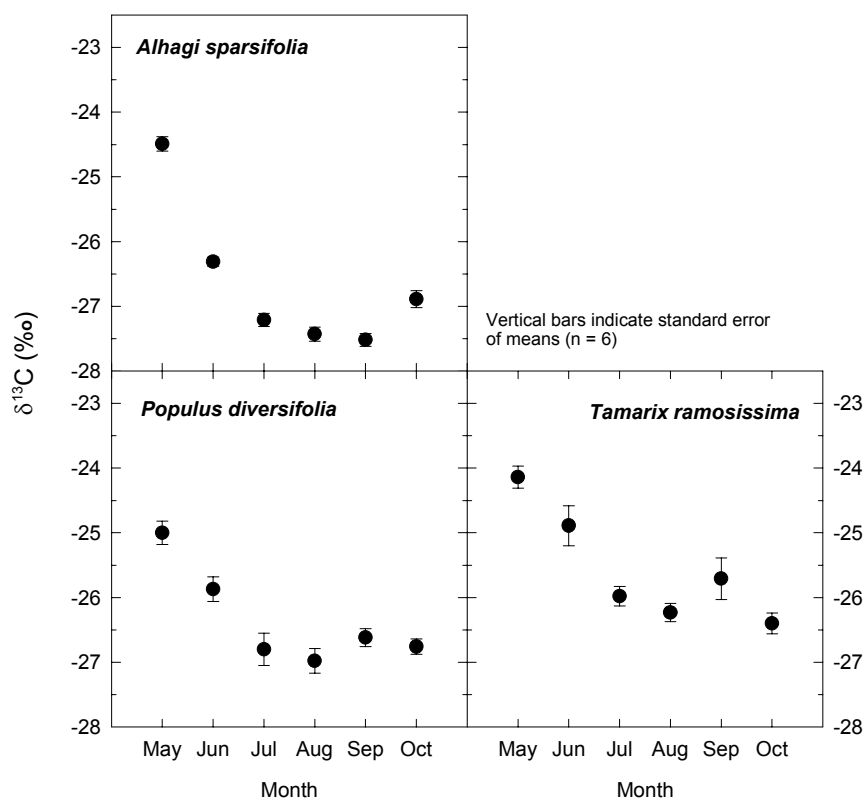


FIG. 1. Annual patterns of  $\delta^{13}\text{C}$  values in three perennial desert species, near Qira Research Station, China.

TABLE I.  $\delta^{13}\text{C}$  VALUES OF LEAVES OF DOMINANT INDIGENOUS SPECIES IN AUGUST 2000 AT QIRA OASIS, XINJIANG

Functional group	$\delta^{13}\text{C}$	SE	n
Total plant community ( $\text{C}_3$ )	-26.4	0.40	8
Total perennial species ( $\text{C}_3$ )	-26.4	0.45	7
Trees ( $\text{C}_3$ )	-26.2	1.00	3
Shrubs ( $\text{C}_3$ )	-26.3	0.53	3
$\text{C}_4$ species	-13.5	0.18	4

Deeply rooted *Ziziphus mauritiana* showed no changes in C-isotope composition during a 100-day rain-free period in Zimbabwe, whereas shallow-rooted peach trees exhibited signs of severe drought stress and a significant increases in leaf  $\delta^{13}\text{C}$  values [4]. Moreover, Stewart et al. [5] have shown that  $^{13}\text{C}$  natural abundance represents a biological expression of environmental conditions integrated over time on a plant-community level, and that it may provide a more meaningful measure of water availability than rainfall data.

From the available stable-isotope data, it can be concluded that all plants had low WUE values, there was no stomatal limitation of photosynthesis, and the plants did not suffer drought stress at any time during the year. These results are consistent with water-relations, transpiration, and sap-flow data (not shown) indicating that all plant species were well supplied with moisture. Moreover, the data reinforce the assumption that all three species were constantly in contact with groundwater resources and are,



therefore, phreatophytic. In the investigated area, groundwater can be found between 3 and 16 m below the surface. It was detected at the *Populus* site at 4.6 m and at the *Tamarix* site at 6.7 m. The groundwater level of the *Alhagi* site was lower than 8 m; it was detected at a nearby well at a depth of 16 m. The roots of *Tamarix* species may descend to 30 m [8], and those of *Alhagi* species to 15 m [9]. *Populus* trees have been reported to be obligate phreatophytes that generally use only groundwater [10]. Consequently, this is an additional indication that all species were in contact with groundwater.

For phreatophytes, survival in arid systems depends exclusively on the capacity to send roots to the permanent water table [11]. Ecologically, this has benefits and disadvantages. The benefits are a prolonged growing season and a permanent nutrient supply due to being independent of rainfall and/or inundation. The disadvantage is difficulty in reproduction and, consequently, in regeneration of the population. Establishment of seedlings is difficult in an extremely arid environment like the Taklamakan desert. Water content of the upper soil layers is very low throughout most of the year, consequently the roots of juvenile phreatophytes may fail to make contact with the water table before wilting. Some species like *Alhagi* and *Populus* circumvent this difficulty by vegetative reproduction with root suckers. In this respect, summer floods may play an important role in the establishment phase, as moisture infiltration of deeper soil layers may prolong favourable conditions for phreatophytic seedlings, thus enabling them to reach groundwater.

### ACKNOWLEDGEMENT

The authors thank the European Commission/BBSRC for funding the research (contract ERB IC18-CT98-0275).

### REFERENCES

- [1] LING, Y., Climatic characteristics and their changing tendency in the Taklimakan Desert, *Die Erde Ergänzungsheft* **6** (1991) 122–132.
- [2] XIA, X., et al., *Desertification and Control of Blown Sand Disasters in Xinjiang*, Science Press, Beijing (1993).
- [3] FARQUHAR, G.D., et al., On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves, *Aust. J. Plant Physiol.* **9** (1982) 121–137.
- [4] ARNDT, S.K., et al., Contrasting adaptations to drought stress in field-grown *Ziziphus mauritiana* and *Prunus persica* trees: water relations, osmotic adjustment and carbon isotope composition, *Aust. J. Plant Physiol.* **27** (2000) 985–996.
- [5] STEWART, G.R., et al.,  $^{13}\text{C}$  natural abundance in plant communities along a rainfall gradient: a biological integrator of water availability, *Aust. J. Plant Physiol.* **22** (1995) 51–55.
- [6] GARTEN JR., C.T., TAYLOR JR., G.E., Foliar  $\delta^{13}\text{C}$  within a temperate deciduous forest: spatial, temporal, and species sources of variation, *Oecologia* **90** (1992) 1–7.
- [7] EHRLINGER, J.R., COOPER, T.A., Correlations between carbon isotope ratio and microhabitat in desert plants, *Oecologia* **76** (1988) 562–566.
- [8] BAUM, B.R., *The Genus Tamarix*, Israel Academy of Sciences and Humanities, Jerusalem (1978).
- [9] WICKENS, G.E., *Ecophysiology of Economic Plants in Arid and Semi-Arid Lands*, Springer, Berlin, Heidelberg, New York (1998).
- [10] SMITH, S.D., et al. *Physiological Ecology of North American Desert Plants*, Springer, Berlin, Heidelberg, New York (1996).
- [11] CANADELL, J., et al., Maximum rooting depth of vegetation types at the global scale, *Oecologia* **108** (1996) 583–595.

# CARBON ISOTOPE SIGNATURES OF LEAF CARBON FRACTIONS: A NEW APPROACH FOR STUDYING SHORT TERM WATER DEFICITS OF PLANTS

S. HEINTEL, W. WANEK, A. RICHTER

Institute of Ecology and Conservation Biology,  
University of Vienna, Vienna, Austria

Natural abundance levels of C isotopes in leaves have been used for 20 years to assess water-use efficiency of plants based on the effects of stomatal limitation on C-isotope discrimination by Rubisco [1]. The approach has been employed, with varying success, to screen crops for enhanced biomass production and water-use efficiency. However, it can neither be used to assess the response of plants to short-term water deficit situations nor to study stress recovery, although these parameters are probably more relevant for the selection of drought-tolerant and highly productive crops [2].

Therefore, our objective with the present study was to develop a method for assaying short-term stress responses by fractionating bulk leaf C into lipids, soluble sugars, starch, and cellulose, and analysing their  $\delta^{13}\text{C}$  values. The method relies on the non-equilibrium of C isotope abundance in fractions of various turnover times. Cellulose and cell-wall C show the slowest turnover and, due to the fact that it constitutes most of the leaf C, they often mask short-term changes in C fractions, such as soluble sugars and starch that undergo rapid synthesis and breakdown. The time-course of  $\delta^{13}\text{C}$  values of various leaf-C fractions of adzuki bean (*Vigna angularis*) during a 7-day drought period are presented.

The soil water content declined significantly after 3 days. As expected, rapid and significant  $^{13}\text{C}$  enrichments were found in soluble sugars and starch, not in cellulose or lipids, in response to water deficit (Fig. 1) Thus, fractionating leaf C and measuring its isotopic composition is a sensitive tool for studying short-term water deficit of plants.

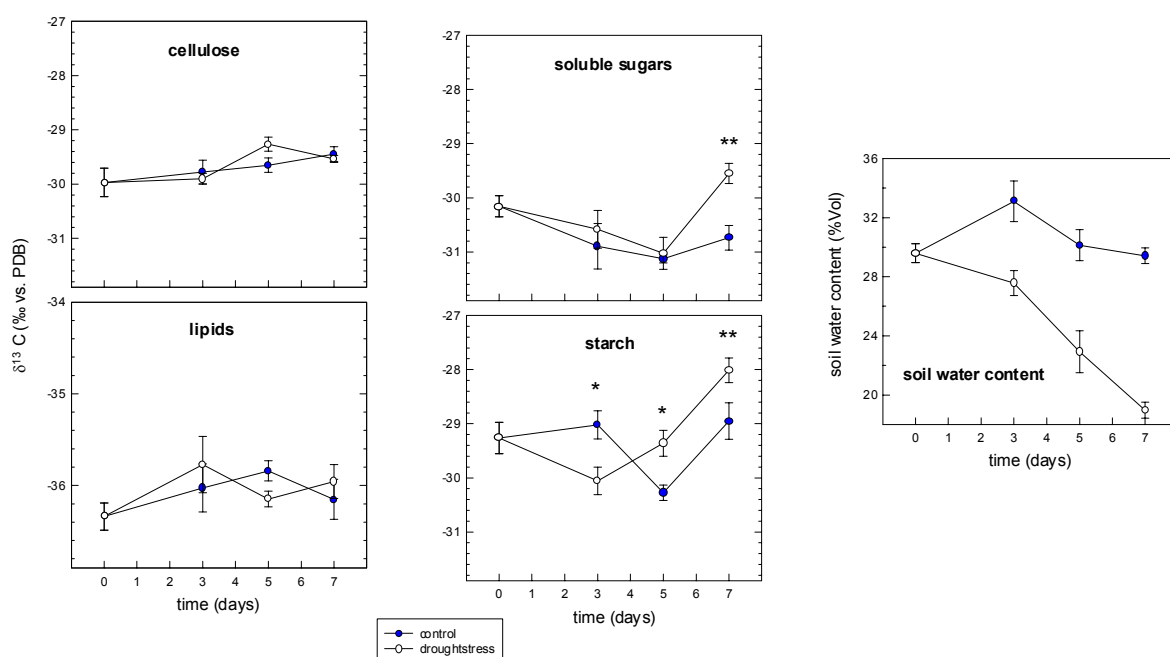


FIG. 1. Drought-stress experiment with *Vigna angularis*.

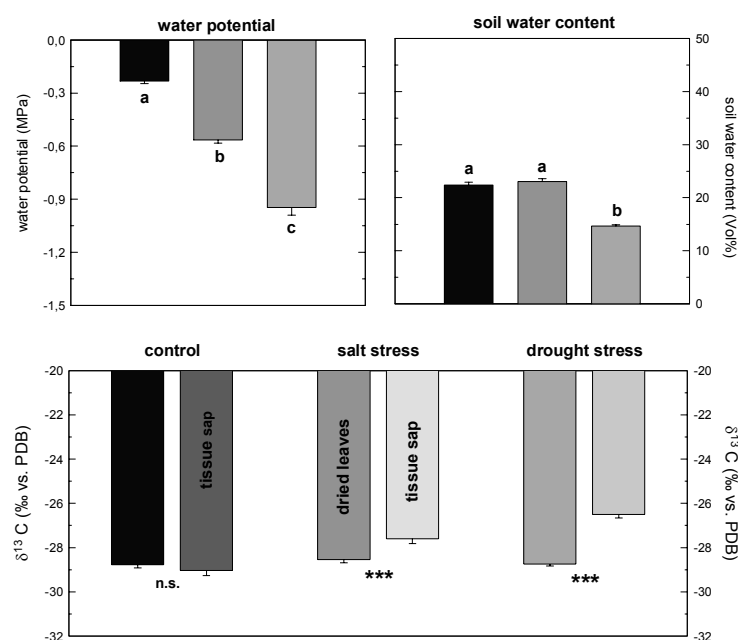


FIG. 2. Stress experiment with *Lycopersicon esculentum*.

Also, a simple field-method was developed, based on the comparison of isotope signatures of bulk leaf C and tissue sap, the latter representing a more active metabolic pool (i.e. soluble carbohydrates). The method was applied to a study of the stress response of drought- and salt-tolerant tomato (*Lycopersicon esculentum*) cultivars.

After 3 days of withholding water (drought treatment) or exposing the plants to 250 mM NaCl (salinity treatment) the  $\delta^{13}\text{C}$  value of the bulk leaf material was not significantly different from that of control plants (Fig. 2). However, the difference between bulk-leaf and tissue-sap C-isotope composition ( $\Delta\delta^{13}\text{C} = \delta^{13}\text{C}_{\text{bulk}} - \delta^{13}\text{C}_{\text{sap}}$ ) was only 0.40‰ in controls, whereas it was -0.95‰ and -2.24‰ in salt and drought stressed leaves, respectively. The differences in  $\Delta\delta^{13}\text{C}$  between controls and both treatments were highly significant ( $P < 0.001$ ), thus proving the suitability and sensitivity of the method for assessing short-term responses to stress.

## ACKNOWLEDGEMENT

We thank the Hochschuljubiläumsfond der Stadt Wien (project H-155) for their support.

## REFERENCES

- [1] EHLERINGER, J.R., et al., Stable Isotopes and Plant Carbon-Water Relations, Academic Press, San Diego (1993).
- [2] BRUGNOLI, E., et al. (1998) "Carbon isotope discrimination in structural and non-structural carbohydrates in relation to productivity and adaptation to unfavourable conditions," Stable Isotopes. Integration of Biological, Ecological and Geochemical Processes (GRIFFITHS, I.H., Ed.), Bios Scientific Publishers, Oxford (1998) 133–146.

# IMPACT OF SOIL MOISTURE ON NODULATION, BIOLOGICAL NITROGEN FIXATION AND YIELDS OF SELECTED TROPICAL LEGUMES

U.R. SANGAKKARA, A.D.A. RATNAYAKE  
Faculty of Agriculture, University of Peradeniya,  
Peradeniya, Sri Lanka

K.S. KUMARASINGHE\*  
Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture,  
International Atomic Energy Agency, Vienna

## ABSTRACT

Field studies were conducted over the dry seasons of two consecutive years to determine the impact of moisture stress on root-nodule number, patterns of N assimilation and grain or biomass yields of ten tropical food, green-manure, and fodder legumes, using  $^{15}\text{N}$ . The plots were either rain-fed, which was insufficient for crop growth or irrigated to maintain soil moisture at 75 to 90% of field capacity. All measured parameters were affected by moisture deficiency; however, nodulation was the least influenced. Species prone to adverse effects from drought were affected to a greater extent than cowpea and pigeon pea, which are tolerant of or resistant to drought. The impact of water stress was greater on biological  $\text{N}_2$  than on yields. The drought-susceptible species extracted a greater proportion of N from soil than from fertilizer.

## 1. INTRODUCTION

Legumes are a vital component of tropical farming systems both in terms of capacity to biologically fix atmospheric  $\text{N}_2$  and in terms of agronomic and economic value due to their ability to produce protein-rich food and fodder under a wide range of environments [1]. Their value is expected to increase further due to their important role in developing sustainable farming systems, especially in the tropics, as biological  $\text{N}_2$  fixation (BNF) is considered vital for maintaining soil fertility [2].

The formation and function of root nodules, in which the BNF occurs, are extremely sensitive to water stress [3]. Hence the productivity of legumes and their capacity for BNF is affected by soil moisture deficits, especially when cultivated under rain-fed conditions in the tropics [4]. The impact of moisture stress is reported to be greater on BNF than on photosynthesis, which governs crop productivity [5].

Legumes vary in the ability to withstand moisture stress, both between and within species [6], due to differences in morphological and physiological adaptation. However, comparative studies have not clearly documented the impact of soil moisture deficits on BNF in tropical food and fodder legumes grown under similar conditions, although water is considered to be the factor most likely to limit tropical crop production [7]. Therefore, a field study was made to determine the effects of soil moisture on nodulation, BNF, and yields of ten tropical food and fodder legumes, grown under field conditions in the dry season over 2 consecutive years.

## 2. MATERIALS AND METHODS

The field study was conducted in the dry seasons (May–August) of 1994 and 1995 at two sites at the Experiment Station of the University of Peradeniya, Sri Lanka. The mean rainfall and pan evaporation during this period are 514 mm and 625 mm respectively, thus subjecting all crops grown under rain-fed conditions to soil-moisture deficits. The mean temperature was 29.6°C with a humidity of 68%.

---

\* Deceased.

The legume species used were *Sesbania rostrata* (sesbania), *Vigna radiata* (mung bean), *Phaseolus vulgaris* (common bean), *Vigna unguiculata* (cowpea), *Psophocarpus tetragonolobus* (winged bean), *Arachis hypogaea* (groundnut), *Crotolaria juncea* (crotolaria), *Cajanus cajan* (pigeon pea), *Desmodium ovalifolium* (desmodium) and *Stylosanthes gracilis* (stylo), with *Elusine coracana* (finger millet) as the non-fixing comparison crop. The soil-moisture regimes imposed were 30 L/plot at 3-day intervals (irrigated) and the absence of supplementary water (non-irrigated). The experiment, which had twenty treatments, was replicated four times within a randomized block design in each season.

The species were planted in well prepared plots of dimensions 4×4 m, having isotope microplots of dimensions 1×2 m. Nitrogen fertilizer (ammonium sulphate) with an <sup>15</sup>N atom excess of 10% was applied to the microplots just prior to planting at a rate equivalent to 40 kg N/ha. The remainder of each plot received the same rate of non-labelled ammonium sulphate. All plots were supplied with 60 kg P and 50 kg K/ha at the same time, and the crops were managed per local recommendations.

At the V8/R1 growth stage of each crop, six plants of each species were carefully excavated from the microplots, roots were washed, and nodules counted. The plants were dried at 80°C for 48 h and weighed. Subsamples were ground and analysed for total N, and <sup>15</sup>N enrichment by mass spectrometry. The soil moisture of each plot to a depth of 40 cm was determined gravimetrically at 5-day intervals from the day of planting until sampling, and mean values determined. At maturity of each food legume, the grain yields were determined on a subplot of 2 m<sup>2</sup>. The green pod yields of common bean and winged bean were also determined as the harvested product. In the fodder legumes and green manures, dry biomass was determined after 5 months in a subplot similar to that harvested for the food legumes.

The data of the two seasons were pooled due to their similarity, and were subjected to appropriate statistical analysis to determine the significance of observed differences and the presence of significant interactions.

### 3. RESULTS AND DISCUSSION

The mean soil-moisture contents of irrigated and non-irrigated plots were 75% to 90% of field capacity and below 50% of field capacity, respectively. Thus, plants grown in the non-irrigated plots were subjected to soil-moisture deficits.

Nodule numbers of all species declined in non-irrigated plots (Table I), with no interaction between species and soil moisture. The reduction was less than 10% in all species with the exception of common bean, a poorly nodulating species (28%) and in desmodium (18%). Therefore, under prevailing conditions, nodule initiation was not significantly affected by soil moisture deficits for most of the species.

Lack of adequate soil moisture had significant effects on patterns of assimilation of N (Table I). Responses varied with adaptability to soil-moisture depletion. In all species, N derived from fertilizer (N<sub>dfF</sub>) increased under low soil moisture. The most significant increases were in pigeon pea, cowpea and stylo, which are relatively well adapted to dry conditions. The smallest increment was in common bean, followed by sesbania, crotolaria, and winged bean. This showed that, under dry conditions, drought-tolerant species have the capacity to exploit fertilizer N more efficiently.

Lack of soil moisture decreased BNF (N derived from air, N<sub>dfA</sub>) in all species, while increasing N derived from soil (N<sub>dfS</sub>). The most significant reduction in BNF was in common bean, a drought-susceptible and poorly fixing species. The smallest reductions in BNF in non-irrigated plots again were in pigeon pea and cowpea. Ranking on the basis of the reduction in BNF due to soil moisture deficit was: common bean > winged bean > desmodium > mung bean > sesbania > groundnut > crotolaria > stylo > cowpea > pigeon pea.

TABLE I. NODULATION AND NITROGEN DYNAMICS OF TROPICAL LEGUMES AS AFFECTED BY SOIL MOISTURE

Species	Moisture regime	Nodules (per plant)	Ndff	Ndfa	Ndfs
			———— (mg N/plant) ————		
Sesbania	Irrigated	84	41.6	275	37.2
	Non-irrigated	78(7) <sup>a</sup>	51.2(24)	215(21)	46.5(24)
Mung bean	Irrigated	126	28.5	164	24.6
	Non-irrigated	115(8)	36.4(28)	126(23)	31.5(29)
Common bean	Irrigated	49	85.4	128	62.5
	Non-irrigated	35(28)	102(18)	62.5(51)	96.2(54)
Cowpea	Irrigated	145	106	426	34.1
	Non-irrigated	131(9)	139(32)	358(15)	42.5(23)
Winged bean	Irrigated	96	186	457	42.8
	Non-irrigated	89(7)	232(25)	306(33)	58.4(38)
Groundnut	Irrigated	127	112	182	46.4
	Non-irrigated	116(8)	149(32)	146(19)	64.8(26)
Crotalaria	Irrigated	174	136	242	40.8
	Non-irrigated	160(8)	169(25)	195(19)	52.6(30)
Pigeon pea	Irrigated	105	115	386	50.8
	Non-irrigated	97(7)	155(36)	352(8)	59.5(18)
Desmodium	Irrigated	47	128	194	45.9
	Non-irrigated	42(18)	162(25)	249(28)	59.0(31)
Stylos	Irrigated	85	95.4	285	60.5
	Non-irrigated	81(4)	124(30)	232(18)	76.1(26)
Probability	Species	0.038	0.029	0.004	0.017
	Irrigation	0.001	0.031	0.002	0.040
	Interaction	0.514	0.009	0.016	0.022

<sup>a</sup>Percent change compared to irrigated counterpart within a species.

Declining BNF in all species was associated with increases in NdfS. The highest uptake of soil N was in common bean, followed by winged bean, neither of which is well adapted to dry conditions. The lowest increment in NdfS was in pigeon pea, which is drought tolerant. An analysis of Ndff and Ndfs illustrated a negative relationship ( $Y = 10^{3.14} \times X^{-1.4}$   $r^2 = 0.641^*$ ), thus indicating the greater use of soil N by species not adapted to dry conditions, due to less utilization of fertilizer N under moisture stress.

The lack of adequate soil moisture reduced yields of all of the legumes (Table II). Declines varied with species, in a manner similar to that for BNF (Table I).

As expected, the impact of soil-moisture deficit was most significant in drought-susceptible species such as common bean and winged bean, which are grown mainly for green pods in the tropics. The impact of moisture stress was also significant in desmodium and mung bean. Drought-tolerant or resistant species, such as cowpea and pigeon pea, were least affected by moisture stress. The effects of soil-moisture deficit on pigeon pea were minimal, confirming its suitability for dryland farming in the tropics.

TABLE II. IMPACT OF SOIL MOISTURE ON YIELDS OF SELECTED TROPICAL FOOD , GREEN MANURE AND FODDER LEGUMES

Species	Moisture regime	Yield (kg/ha)
Sesbania <sup>a</sup>	Irrigated	3,420
	Non-irrigated	2,804(18%) <sup>b</sup>
Mung bean	Irrigated	2,948
	Non-irrigated	2,357(20%)
Common bean	Irrigated	4,242
	Non-irrigated	2,331(45%)
Cowpea <sup>c</sup>	Irrigated	2,241
	Non-irrigated	1,974(12%)
Winged bean <sup>c</sup>	Irrigated	4,426
	Non-irrigated	3,151(29%)
Groundnut	Irrigated	3,425
	Non-irrigated	2,875(16%)
Crotalaria*	Irrigated	4,210
	Non-irrigated	3,491(17%)
Pigeon pea	Irrigated	1,942
	Non-irrigated	1,840(5%)
Desmodium <sup>a</sup>	Irrigated	1,846)
	Non-irrigated	1,411(23%)
Stylo <sup>a</sup>	Irrigated	2,046
	Non-irrigated	1,735(15%)
Probability	Species	0.041
	Irrigation	0.002
	Interaction	0.035

<sup>a</sup>Dry herbage yields. <sup>b</sup>Percentage decrease compared to irrigated counterpart within a species. <sup>c</sup>Green-pod yields.

It may be inferred from the data that nodulation per se is not significantly affected by soil-moisture stress. Nodule function, as indicated by NdfA values, was more adversely affected than nodule number. In addition, species that are adapted to dry conditions increased uptake of applied N, to maintain N balances rather than exploit soil N. In contrast, species such as common bean, winged bean and sesbania, which require adequate soil moisture to yield well, tended to lower BNF significantly, while increasing the uptake of soil N rather than applied fertilizer. This would remove significant quantities of N from the soil, leading to the loss of sustainability.

The effect of moisture stress on yield, although similar to that on BNF, was, importantly, less extreme. Clearly the effect on root-nodule symbiosis was greater than that on yield, which is governed by photosynthesis and other physiological processes. This is an important concept hitherto not presented for various legumes on a comparative basis.

#### 4. CONCLUSIONS

Farmers who plant legumes in the dry season under rain-fed conditions for food or fodder should be encouraged to grow species that are adapted to lower soil-moisture conditions to minimize the loss of nitrogen from the ecosystem and to maintain BNF and procure high yields. If species such as common bean are grown in the dry season, they need to be provided with supplementary irrigation to prevent the uptake of N from the rhizosphere. This would enable these species to grow successfully and produce higher yields to procure greater yields and also add more biomass in terms of crop residue for soil enrichment.

#### ACKNOWLEDGEMENT

The authors thank the University of Peradeniya for research funds.

#### REFERENCES

- [1] SWIFT, M.J., WOOPER, P., “Organic matter and sustainability of agricultural systems”, *Soil Organic Matter Dynamics and Sustainability of Tropical Agriculture* (MULONGOY, K., MERCKX, R., Ed.), Wiley and Sons, London (1993) 3–18.
- [2] SERRAJ, R., et al., Symbiotic N fixation response to drought, *J. Exp. Bot.* **50** (1999) 143–155.
- [3] KIRDA, C., et al., Temporal water stress effects on nodulation, nitrogen accumulation and growth of soybean, *Plant Soil* **120** (1989) 49–55.
- [4] SUBBARAO, G.V., et al., Strategies for improving drought resistance in legumes, *Crit. Rev. Pl. Sci.* **14** (1995) 469–523.
- [5] WERY, J., et al., Screening techniques and sources of tolerance to extremes of moisture and air temperature in cool season food legumes, *Euphytica* **73** (1994) 73–83.
- [6] del ROSAIRO, D.A., “Adaptation of vegetable legumes to drought stress”, *Adaptation of Food Crops to Temperature and Water Stress* (KUO, C.G., Ed.), AVRDC, Taiwan (1993) 360–370.
- [7] BOUWER, H., Integrated water management: emerging issues and challenges, *Agric. Water Mgt.* **45** (2000) 217–228.





# **ENVIRONMENTAL AND POLLUTION STUDIES**

(Session 5)



**Keynote Address****USE OF NUCLEAR TECHNIQUES IN ENVIRONMENTAL AND POLLUTION STUDIES**

M.H. GERZABEK, G. HABERHAUER, A. KRENN, T. SHINONAGA  
 Department for Environmental Research,  
 Austrian Research Center, Seibersdorf, Austria

**Abstract**

We present a brief review of nuclear techniques used in various research endeavours in the environmental sciences, with special emphasis on the soil-plant system. The following topics are described and exemplified: (i) the present use of neutron activation analysis with emphasis on soil-to-plant transfer studies, (ii) the application of the isotope-dilution approach in heavy-metal adsorption studies in soil, (iii) the use of tracer techniques to follow the fate of organic pollutants in the soil-plant-groundwater system, and (iv) the determination of natural abundances of stable isotopes of N and O in studies on the fate of deposited nitrate in a forest ecosystem.

**1. INTRODUCTION**

For decades, nuclear techniques have seen widespread use, for a variety of purposes, in the agricultural sciences. Studies in nutrient use and turnover, for example, have benefited greatly from the employment of isotopes. Similarly, the environmental sciences profit from the advantages of radioactive or stable tracers and other nuclear analytical techniques such as neutron activation analysis: low detection limits allow traces to be quantified and, therefore, pool sizes and fluxes between pools can be evaluated. Thus, there is great potential for the assessment of medium- and long-term behaviour of environmental pollutants. The main advantage of nuclear methods in many cases is high specificity, which often implies the status of a reference method in the respective field. The main nuclear techniques used in environmental research are briefly reported in the following three sections (see also Refs. [1,2,3]). In sections 5 and 6, some applications of nuclear methods in soil and soil-plant studies are exemplified.

**2. NUCLEAR ANALYTICAL TECHNIQUES USED IN THE ENVIRONMENTAL SCIENCES**

Nuclear methods are frequently used when high specificity and sensitivity are required. Some nuclear techniques, e.g. X ray fluorescence (XRF), particle-induced X ray emission (PIXE) and nuclear activation analysis (NAA), have particular advantages for highly complex environmental samples, like soil and plant materials. In cases where total elemental content of environmental samples is to be determined, conventional techniques like optical atomic spectrometry (e.g. atomic absorption spectroscopy, AAS), inductively coupled plasma optical emission or mass spectrometry (ICP-OES, ICP-MS) involve dissolution steps prior to analysis. In many cases, dissolution does not yield the total element content, especially in cases such as soil samples that contain large quantities of mineral matter [4,5]. The direct determination of elements in a sample minimizes risks of cross-contamination that sometimes occurs during laborious digestion procedures. The conventional analytical methods mentioned above have advantages when physico-chemical properties of pollutants are to be investigated, due to the fact that they are more convenient for the analysis of liquid samples. The following techniques are used for analytical purposes:

- radiochemical techniques [neutron activation analysis (NAA), isotope-dilution methods, radio-release and -displacement methods, etc.],
- radiometric methods (absorption and scattering of ionizing radiation, X ray fluorescence, particle-induced X ray emission, etc.),
- trace-gas analysis by ionization detectors.

Neutron activation analysis in environmental studies was recently reviewed by Steinnes [6]; it became popular as nuclear reactors became increasingly available in the 1950s and 1960s. Very small sample volumes, low detection limits for up to fifty elements, multi-element detection, and applicability to various environmental sample categories, such as soils, plants [7], aerosols, filters, coal, and water, made NAA attractive. Table I provides an overview of detection limits of NAA using thermal neutrons (reactor neutrons).

In the past, NAA was the most sensitive multi-element analytical method. However, ICP-OES and, recently, ICP-MS have become universal for environmental analyses. The latter, especially, has low detection limits, comparable to, or even lower than, instrumental NAA (INAA) [6]. Another disadvantage of NAA is the declining availability of reactors for irradiation purposes. A major advantage of ICP-MS is the fact that environmentally important elements, e.g. Cd, Cu, Ni, and Pb, can easily be determined, whereas this is difficult with INAA. According to Steinnes [6] the major areas of NAA application in the future will be (i) analysis of small samples (aerosols, size fractions of materials, etc.), (ii) analysis of samples difficult to decompose, (iii) analysis of elements difficult to determine by other methods (e.g. halogens, see section 5) and (iv) certification of reference materials.

X ray fluorescence techniques are the most popular of the analytical radiometric methods. Multi-element analysis and the possibility of direct sample measurements again are the major advantages. Particle-induced X ray emission spectroscopy (PIXE) is gaining importance in the environmental sciences, due to low detection limits and small sample size, down to the individual particle level ( $\mu\text{m}$ -sized particles; microPIXE). Compared to INAA, XRF and PIXE are more likely to be subject to matrix effects, but speed of analysis is distinctly higher [3]. Thus, a major field of application is the analysis of atmospheric aerosols as a component of studies of local air pollution, and short-, medium- and long-range transport of pollutants.

X ray absorption methods like X ray absorption fine structure spectroscopy (XAFS) and near-edge X ray fine structure absorption spectroscopy (NEXAFS) presented new opportunities in soil research, such as the study of kinetics of metal sorption on minerals, shown recently [8]. Studies with Zn and Ni showed that these metals form mixed precipitates with Al-hydroxide. Carbon-edge X ray spectromicroscopy allows characterization of soil organic matter in situ [9]. The average local bonding environment of C atoms in both solid and aqueous species can be resolved with NEXAFS.

TABLE I. DETECTION LIMITS OF NEUTRON ACTIVATION ANALYSIS USING REACTOR NEUTRONS (MODIFIED FROM REF. [1])

Detection limit (g)	Element
$10^{-15}$	Eu
$10^{-14}$	In, Dy
$10^{-13}$	Mn, Sm, Ho, Lu, Re, Ir, Au
$10^{-12}$	Na, Ar, V, Co, Cu, Ga, As, Br, Kr, Rh, Pd, Ag, I, Cs, La, Pr, Yb, W
$10^{-11}$	Al, Cl, K, Sc, Ge, Se, Y, Sb, Xe, Ba, Gd, Tb, Er, Tm, Pt, Os, Hf, Hg, Th, U
$10^{-10}$	Si, P, Cr, N, Zn, Sr, Nb, Ru, Cd, Sn, Tc, Ce, Nd, Ta
$10^{-9}$	F, Ne, Mg, Ti, Rb, Mo, Tl, Bi
$10^{-8}$	S, Ca, Zr, Pb
$10^{-7}$	Fe
$10^{-4}$	N, O

Nuclear methods are widely used in routine analytical procedures. For example, dust analysers for monitoring purposes use the principle of  $\beta$ -radiation absorption for quantification of dust emissions. Gas-ionization detectors are widely used to analyse trace gases, especially in gas chromatography. Even pesticides and compounds containing sulphur and metal-organic complexes can be selectively analysed in traces [1].

### 3. ISOTOPE DILUTION AND HEAVY-METAL AVAILABILITY IN SOIL

One of the most challenging issues in the environmental sciences is the determination of mobile fractions of pollutants in soil. These measurements are urgently needed for calculations and modelling of pollutant fluxes. Knowing the mobile fraction in soil facilitates the prognosis of entry into the food chain of heavy metals and radionuclides, for example. It has been shown, that isotopically exchangeable Zn in disturbed soil samples is strongly related to isotopically exchangeable Zn determined in pot experiments [10]. Recently Sinay et al. [11] developed a method for a short-term isotope-exchange kinetic study using  $^{65}\text{Zn}$  to predict steady-state concentrations of Zn in the soil solution. Predictions for a 3-day equilibrium phase were made from 100-min experiments. The important advantage of the isotope-exchange kinetics approach is the fact that all three relevant values can be determined:

- the intensity (activity of free ions in soil solution),
- the quantity (ions that can be released from the soil matrix = quantity of isotopically exchangeable Zn), and
- the buffering capacity (changes in quantity per unit change in concentration of metals in solution).

Another advantage is the very low concentration of the selected metal, which prevents bias to the results. Smolders et al. [12] determined the labile Cd pool, using  $^{109}\text{Cd}$ , in small quantities of non-polluted and Cd-polluted soils. It could be shown that Cd-fixation in the soils investigated (“ageing”) was not pronounced, if any was detectable at all.

A closely related approach to characterize the future behaviour of metals in soil is the determination of sorption isotherms. Schug et al. [13] used  $^{109}\text{Cd}$  to assess the influence of initial Cd concentration of the soil on its sorption behaviour, and compared the results with the traditional method. It was concluded that the radioanalytical method for estimating sorption isotherms provides a mechanism of correction concerning the initial Cd contents in soil, which cannot be accomplished using conventional correction procedures (e.g. based on the EDTA-extractable Cd portion). Log-log plots of sorption isotherms measured by means of the radioisotope approach turned out to be almost linear, even in the low-concentration range. Thus, radioactive tracers are promising as a tool for study of the exchangeability of cationic pollutants, especially heavy metals.

### 4. TRACER TECHNIQUES IN THE SOIL-PLANT SYSTEM

In addition to the quantification of pollutants in the environment, radioactive and stable isotopes are used extensively to study the processes involved—which are of at least equal importance—e.g. decomposition, metabolism, and transfer mechanisms among compartments of an ecosystem. Major advantages of tracer applications are as follows:

- high specificity and, thus, low detection limits, for pollutants, which are especially needed for investigations of organic compounds, and
- the potential for investigation of small fluxes between large element pools, which is scarcely possible using conventional methods.

Isotopes prominently used for tracer purposes are,  $^2\text{H}$ ,  $^{13}\text{C}$ ,  $^{14}\text{C}$ ,  $^{15}\text{N}$ ,  $^{18}\text{O}$ ,  $^{34}\text{S}$ , and  $^{37}\text{Cl}$ . The main topics investigated in the soil and plant sciences are (i) the behaviour of pesticides or other organic pollutants in soil and their transfer to plants and groundwater, (ii) losses of N fertilizers and their impact on groundwater pollution, and (iii) the impact of environmental stresses on plants and their physiological reactions.

The behaviour of pesticides in the soil-plant system is complex [14]. Meteorological conditions, soil and plant characteristics, application techniques, and the physical and chemical properties of the applied compound itself govern its environmental behaviour. A major problem is quantification of mineralization, metabolism, and of persistent “bound residues” in soil. It is particularly necessary to apply tracer techniques when the bound residues are defined [15] as those “compounds in soils, plants or animals that persist in the matrix in the form of the parent substance or its metabolite(s) after extraction; the extraction method must not substantially change the compounds themselves or the structure of the matrix.” According to the type of compound and the soil properties, various types of soil-pesticide interactions are possible. In a recently published review, Gevaio et al. [16] described the following as mechanisms already reported in the literature: ionic binding, hydrogen bonding, van der Waal’s forces, ligand exchange, charge-transfer complexes, hydrophobic partitioning, covalent bonding, and sequestration. A complete picture can be gained by using the lysimeter approach. Several studies have been reported already, in which  $^{14}\text{C}$ -labelled organic compounds were used to elucidate the fate of pesticides. Recently Burauel and Führ [17] summarized results of long-term lysimeter experiments investigating various pesticides. Table II shows large differences among compounds with respect to long-term behaviour in a single soil type. From the presented results it can be concluded that the forms of interaction of organic compounds, which can be envisaged for the presented substance classes, influence microbial turnover due to differences in accessibility to micro-organisms. On the other hand, soil microflora plays an important role in the formation of bound residues due to metabolism of the original compound. For example, metabolites of polycyclic aromatic hydrocarbons (PAHs) can undergo oxidative coupling to phenolic compounds and subsequently form non-hydrolyzable macromolecules that are similar to humic substances [18]. The latter-mentioned bound residues amounted to 45% of the applied compound after 176 days of incubation.

Similar soil-bioreactor experiments with PAHs have been performed using  $^{13}\text{C}$ -labelled compounds, e.g. [ $1\text{-}^{13}\text{C}$ ]phenanthrene [19]. For the detection of  $^{13}\text{C}$ , both isotope ratio MS (IRMS) and  $^{13}\text{C}$ -nuclear magnetic resonance spectroscopy (NMR) can be used. The latter method has the advantage that additional structural information about the bound residues can be gained. For the quantification of bound residues, IRMS is needed due to weakness in the detection limits of NMR-spectroscopy [20].

The use of  $^{13}\text{C}$  and  $^{14}\text{C}$  as tracers for the investigation of the dynamics of organic pollutants in the soil-plant-groundwater system can be further developed by combining the tracer approach with additional physical and/or chemical soil fractionations, as already reported [17,20]. Recently, Benoit and Preston [20] demonstrated the close relationship between bound-residue formation and decomposition of plant residues using a particle-size fractionation procedure. Composting of straw prior to application to soil increased the formation of bound atrazine residues, as compared to soil amended with fresh straw.

Besides the extraction of soil-bound residues by means of alkaline extraction and subsequent extraction with organic solvents, supercritical fluids, high-temperature extraction or microwave extraction, silylation can also be considered to release soil-bound residues of  $^{14}\text{C}$ -labelled xenobiotics [21].

Chemical derivatization of functional groups is of potential interest in connection with the characterization of soil-bound residues, because it:

- makes the characterization of specific functional groups easier,
- reduces the polarity of humic substances and makes them soluble in organic solvents, and
- reduces interactions caused by hydrogen bonding, which facilitates the application of NMR and IR spectroscopy [21].

For silylation purposes, trimethylchlorosilane, N-methyl-N-trimethylsilyltrifluoroacetamide, or similar compounds, are used to substitute reactive hydrogens of functional groups present in organic compounds. Extractability of soil-bound residues in most cases is higher during silylation as compared to alkaline or organic extractions.

TABLE II. BALANCE OF RADIOACTIVITY FROM LYSIMETER EXPERIMENTS WITH  $^{14}\text{C}$ -LABELLED PESTICIDES AND ORGANIC POLLUTANTS (MODIFIED FROM REF. [17])

Compound	Duration of the study (years)	Radioactivity detected (%)	
		Total soil <sup>a</sup> profile 0–110 cm	Non-extractable residues 0–30 cm
Methabenzthiazuron	6.5	20	17
Terbuthylazine	2	66	38
Dichlorprop-P	2	19	18
Benzo(a)pyrene	2	—	18
PCB 28, PCB 52	2	—	16

<sup>a</sup>Orthic luvisol (topsoil pH 7.2, 1.2% C<sub>org</sub>, sand:silt:clay (%) = 6.4:78:15.

The stable isotopes  $^{12}\text{C}$  and  $^{13}\text{C}$  are frequently employed in plant physiological studies that address environmental questions. In many cases,  $^{13}\text{C}/^{12}\text{C}$  ratios are used as a measure of plant stress. Aside from the direct influence of water stress on crops [22], stable C isotopes are useful for investigating stresses on plants influencing the water balance, e.g. UV-B effects on growth inhibition [23] or the photosynthate partitioning by  $\text{O}_3$  action using the pulse-labelling technique [24] including rhizodeposition of organic C [25].

## 5. EXAMPLES: USE OF NEUTRON ACTIVATION IN SOIL-TO-PLANT TRANSFER STUDIES

### 5.1. Soil-to-plant transfer of stable iodine

Iodine is widespread at low concentrations in various compartments of the environment (earth's crust,  $0.30 \text{ mg kg}^{-1}$ ; hydrosphere,  $0.06 \text{ mg kg}^{-1}$ ; biosphere,  $0.05 \text{ mg kg}^{-1}$  [26]). An essential element for humans and animals, the World Health Organisation (WHO) recommends a daily intake of 100 to 150  $\mu\text{g}$  per adult. The main nutrient sources of iodine are sea foods and milk products. Vegetables are also a possible source. Artificial radioiodine, such as  $^{129}\text{I}$  and  $^{131}\text{I}$ , is released from nuclear facilities into the environment. Due to the long half-life of  $^{129}\text{I}$ , the level of this nuclide is increasing and it is expected to exist in the environment, as does stable iodine, over a long time scale [27]. The pathways of radionuclides from nuclear facilities to humans are described in ecological models by transfer between compartments of the environment. An important pathway of iodine entry to the food chain is root uptake by plants. Information about soil-to-plant transfer is not extensive, both due to the few studies conducted with  $^{129}\text{I}$  and to analytical problems connected with the determination of stable iodine, particularly in plant material. As already stated, NAA is especially powerful for the determination of halogens. In our study, we used INAA for analysis of iodine in plant samples, as described in detail elsewhere [28].

We used ICP-MS to analyse forty arable soils in Austria, and observed a range of 1.1 to  $5.6 \text{ mg I kg}^{-1}$  soil for stable iodine, i.e. values typical for areas far from the sea [29]. To meet the required low detection limits, radiochemical NAA was used for cereal samples that are much lower in iodine than the corresponding soil. About 1 g per plant sample was used for irradiation for 5 min in the  $\text{B}_4\text{C}$  irradiation facility of the ASTRA reactor at the Austrian Research Centre, Seibersdorf, or 20 to 25 min in the TRIGA reactor of the Atomic Institute of Universities in Austria. The irradiated samples were then dissolved using alkaline and acid solutions and finally iodine was precipitated as  $\text{PdI}_2$ . Iodine-128 was determined by high-resolution gamma-ray spectroscopy on Ge(Li)-detectors at 442.9 keV. The transfer factor (TF) values were calculated as follows:

$$TF = \frac{\text{iodine concentration in dry plant part (mg kg}^{-1}\text{)}}{\text{iodine concentration in the upper 0.2 m (mg kg}^{-1}\text{)}}$$



Iodine concentrations in cereal grains ranged from 0.002 to 0.03  $\mu\text{g g}^{-1}$  DM. The arithmetic average and the median of all cereal grains were 0.0061 and 0.0046, respectively. The iodine concentrations in most of the cereal-grain samples were  $<0.01 \mu\text{g g}^{-1}$ . Higher values of 0.011, 0.030, and 0.025  $\mu\text{g g}^{-1}$  iodine were found in two winter wheat samples and in one winter rye, respectively.

The values obtained in this study showed that the variation in iodine concentration among cereal types, was not large, nor is it among locations in Austria.

The TF for iodine ranged from 0.0005 to 0.017 and the arithmetic average and median were 0.0029 and 0.0016, respectively (Table III). Our TFs of  $^{127}\text{I}$  for cereal grains cultivated in Austria were comparable to the value for grass reported by Frissel and van Bergeijk [30], whereas they were much smaller than those reported by Ng [31] and Robens et al. [32]. Our values are comparable to those for rice grains (polished), approximately 0.002, reported by Muramatsu et al. [33].

The correlation coefficient between iodine content and clay content in soil obtained in this study was  $r=0.75$  ( $P < 0.001$ ). Thus the larger the fraction of clay minerals, the higher was the iodine concentration. Anion sorption occurring at localized positive charges appearing on (i) free hydroxides of Fe and Al, and (ii) the edges of the alumino-silicate clay mineral lattices where the O atoms are not fully co-ordinated by Al or Si atoms, might be the explanation for the observed phenomenon [34,35]. A compilation [35a] of Kd values demonstrated that soils rich in clay and organic matter have higher retention potential for iodine than do sandy or loamy soils. This effect was also reflected by the TFs, which decreased with increasing clay contents of soils ( $r = -0.78$ ,  $P < 0.001$ ).

A correlation between iodine and soil organic matter content, indicated the literature [35], was confirmed in this study by a slightly positive correlation. However it could not be confirmed that iodine is associated with organic C, because organic matter contents in Austrian soils are low and other factors (e.g. clay content) may be contributory. There was no clear relationship between TF into cereal grains and organic matter contents in soil. Some studies with iodide indicate that sorption and desorption of iodine in soil is influenced by pH. It is known that iodine sorption on soil increases with decreasing pH in the soil-water system [36]. The results obtained in this study, however, indicate that the correlation between TF and pH values of 5.4 to 7.6 of Austrian soils was not as clear as with clay mineral content. From our results and those of Muramatsu et al. [37], it may be assumed that pH played a less important role for TF of iodine whereas the clay content, and other possible factors, e.g. aluminium and iron oxides, contributed more strongly.

## 5.2. NAA for estimation of soil contamination on plant surfaces

Soil adhering to plant surfaces is a potential human risk through ingestion. Due to known differences of mobility of pollutants contained in plant tissues or fixed to soil particles, and resulting absorption into the human or animal gut, it is important to know the respective mass-loading of plant surfaces.

TABLE III. TRANSFER FACTORS FOR IODINE INTO CEREAL GRAINS; IODINE CONTENTS IN GRAINS AND SOILS (TOPSOIL 0–0.2 m) IN AUSTRIA (MODIFIED FROM REF. [26],  $n = 38$ )

	Transfer factor of iodine	I in cereal ( $\mu\text{g g}^{-1}$ )	I in soil ( $\mu\text{g g}^{-1}$ )
Arithmetic mean	0.0029	0.0061	3.13
Standard deviation	0.0034	0.0056	1.39
Median	0.0016	0.0046	3.21
Geometric mean	0.0019	0.0050	2.80
Minimum	0.0005	0.0022	1.08
Maximum	0.017	0.0300	5.63

Large variations in soil-to-plant transfer factors used in dose-assessment models may also result from soil contamination of plant surfaces [38]. Soil particles are transferred to plant surfaces by rain-splash and wind erosion. There are three methods for estimating soil mass-loading: (i) ultrasonic washing to treat the vegetation and measurement of plutonium content in particles in the wash water [39], (ii) measurement of the titanium content of plant material after removing iron [40], and (iii)  $^{238}\text{Pu}$  as a monitor of soil transport to plants [41]. The latter method is not useful if  $^{238}\text{Pu}$  content of the soil is very low; additionally a chemical separation is involved, as is the case with the titanium method, which may introduce further uncertainties.

Scandium is geologically ubiquitous in soils, but scarcely absorbed by plant roots and not mobile within plant tissues. Therefore, Sc was chosen as a tracer for soil mass-loading and a  $^{46}\text{Sc}$  NAA method was developed. The physical half-life, 84 days, allows the decay of the large majority of radionuclides, which results in a low Compton background. No chemical separations are needed. A detection limit of  $0.05 \text{ mg soil g}^{-1}$  dry plant biomass was obtained using sample masses of 0.1 g of soil and 1.5 g of dried plant samples [42].

In a combined greenhouse and field experiment, the effects of wind erosion and rain-splash were investigated using ryegrass and broad bean as test species. Plants were grown (i) in the greenhouse in pots in a closed chamber, (ii) in the field with polyethylene film coverage of the soil surface to avoid rain-splash and (iii) in the field without any protection. In Table IV, the main results of this experiment are presented together with literature data.

TABLE IV. SUMMARY OF SOIL MASS LOADING ON PLANT SURFACES FROM THE LITERATURE AND OUR OWN MEASUREMENTS

Plant	Pathway	mg soil $\text{g}^{-1}$ DM	Number of observations	Ref.
Grass	n.s. <sup>a</sup>	$18 \pm 48$	26	[39]
Tomatoes	n.s.	17		[43]
Lettuce	n.s.	$260 \pm 100$	4	[44]
Broccoli	n.s.	$10 \pm 8.1$	4	[44]
Turnips	n.s.	$32 \pm 11$	4	[44]
Cabbage	n.s.	$1.1 \pm 1.1$	4	[44]
Tobacco	n.s.	$2.1 \pm 0.6$	12	[45]
Sunflower	n.s.	$2.6 \pm 0.9$	10	[46]
Soybean	n.s.	2.1	10	[41]
Wheat	n.s.	4.8	10	[41]
Corn	n.s.	1.4	10	[41]
Broad bean	wind erosion	3.0	4	[42]
	rain-splash	6.5	4	[42]
	total	9.5	4	[42]
Ryegrass	wind erosion	3.1	4	[42]
	rain-splash	2.7	4	[42]
	total	5.8	4	[42]

<sup>a</sup>Not specified.

Soil retained on plant surfaces under field conditions was 5.77 mg soil g<sup>-1</sup> DW for ryegrass and 9.51 for broad bean. The estimated contributions of soil splash and wind erosion were 68% and 32% for broadbean and 47% and 53% for ryegrass, respectively. The differences might be due to plant anatomy and leaf structure.

As the soil-to-plant TF does not discriminate between outer plant contamination and root uptake, the soil mass-loading concept might be used for correction purposes applying the following formula:

$$A_r = A_t - A_s \times M_s \times 0.001 \quad (1)$$

where

$A_r$  is the activity concentration in plant tissue due to root uptake (Bq kg<sup>-1</sup>),

$A_t$  is the total activity concentration in the plant (Bq kg<sup>-1</sup>),

$A_s$  is the activity concentration in the soil (Bq kg<sup>-1</sup>), and

$M_s$  is the soil mass-loading value (mg soil g<sup>-1</sup> plant).

Applying the above method to a sample set from Austrian soil-to-plant TF investigations under field conditions, it could be shown the contribution of mass-loading to the <sup>137</sup>Cs contamination of cereal straw samples and the respective TFs ranged from 3 to 23%. This effect was due to soil mass-loading values of 1.1 to 6.0 mg soil g<sup>-1</sup> plant [42]. The respective corrected TFs ranged from 0.008 to 0.057. Considering other inorganic or organic pollutants with similar or even less availability for root uptake, the described mass-loading concept might be of at least equal significance.

## 6. EXAMPLES: USE OF RADIOACTIVE AND STABLE TRACERS IN ENVIRONMENTAL STUDIES

### 6.1. Ozone effects on pesticide degradation in soil

Carbon-14 labelling, a powerful method for elucidating the fate of persistent organic pollutants in the soil-water-plant system, is widely used in laboratory, wind-tunnel and lysimeter experiments [17] (see section 4). It is valuable also for detailed investigation of the fate of organic pollutants in the environment. Tropospheric ozone is regarded as one of the most important phytotoxic air pollutants. The annual mean concentration of ozone in the northern hemisphere has increased over the past 100 years. While tropospheric ozone shows a negative effect on plants and micro-organisms, high concentration of ozone can be used as an oxidant for elimination of pesticide waste [47]. The aim of our study was to examine a possible contribution of elevated concentrations of ozone in air on the degradation of organic pollutants in soil, using dichlorprop [2, 4- Dichlorophenoxypropionic acid (2,4 – DP)] as an example [48]. Dichlorprop belongs to the group of phenoxyalkanoic acid herbicides such as 2,4-D, MCPA, and MCPP. Important as broad-spectrum, pre- and post-emergent herbicides, they are extensively used in agriculture.

A soil originating from an extensively used agricultural area northeast of Vienna was chosen for this experiment. The Calcic Chernozem consists of more than 50% silt and 25% clay, and exhibits an organic C content of 2.1%. Carbon-14-ring labelled 2,4-dichlorophenoxypropionic acid (dichlorprop) was used. The specific radioactivity of the pesticide was 34.4 kBq mg<sup>-1</sup>. A stock solution of 15 mg dichlorprop L<sup>-1</sup> was prepared and added to flasks, each containing 50 g of soil, corresponding to an application of approximately 2.25 kg pesticide ha<sup>-1</sup>. The moisture was readjusted to 40% of the maximum water-holding capacity and the soils were aerobically incubated for a total of 32 days at 21±1°C. The air contained 80 nL L<sup>-1</sup> (± 10 nL L<sup>-1</sup>) of ozone, which is regularly found during summer months around highly congested areas and, therefore, is realistic for agricultural sites of the temperate zone. The controls were exposed the same flow rate of ambient air, i.e. an ozone concentration of 12 nL L<sup>-1</sup>. Solutions of KOH were used to trap evolving <sup>14</sup>CO<sub>2</sub>. The radioactivity of the total soil was determined by oxidation in a sample oxidizer followed by liquid scintillation counting (LSC) of the trapped <sup>14</sup>C. Additionally, soil was extracted with acetone and subsequently with water-saturated n-butanol. The combined extracts were measured for total radioactivity by LSC, concentrated and characterized by thin layer chromatography (TLC).

After organic solvent extraction, each 5-g aliquot of soil sample was mixed with 10 mL of 0.1 M  $\text{Na}_4\text{P}_2\text{O}_7$  solution to extract humic substances. The  $\text{Na}_4\text{P}_2\text{O}_7$  solutions were analysed by LSC. The remaining soil was analysed by oxidation to  $\text{CO}_2$  in a sample oxidizer followed by LSC of the trapped  $^{14}\text{C}$  to obtain the non-extractable residues.

After 4 days of incubation, most of the applied radioactivity was still detected in soil. Eight days after adding dichlorprop, the recovered radioactivity in total soil decreased to approximately 60%. After 32 days only 36% and 38% of the radioactivity was recovered in the control and ozone-exposed soil, respectively. The delayed initiation of mineralization is typical for xenobiotics when applied for the first time. This lag phase, which is known for 2,4-dichloro phenoxyalkanoic acids and is commonly explained by a lack of adaptation of soil micro-organisms, becomes shorter with repeated applications [49]. Concerning the total radioactivity in soil, no significant difference was obtained for the ozone samples in comparison to the controls. The  $^{14}\text{C}$  content of the organic extractable fractions behaved similarly to the total  $^{14}\text{C}$ -concentration in the soil.

The concentration of dichlorprop dropped to 64 and 76% after 4 days for control and ozone variants, respectively, and to 17% after 8 days for both variants (Fig. 1). Compared to mineralization, the lag phase was shorter, due to the typical metabolism of dichlorprop, which shows a retarded  $\text{CO}_2$  release [50]. Degradation half-lives of dichlorprop of approximately 5 days were obtained for both treatments. These fit into the range of other published half-lives of dichlorprop obtained for different soils from 3 to 10 days [51,52]. No significant influence of ozone addition to the air stream was observed on the degradation rate of dichlorprop. Up to 7% of the applied radioactivity were incorporated in the sodium-pyrophosphate extract, associated with humic substances, after 32 days. This is low in contrast to  $^{14}\text{C}$  bound to non-extractable soil constituents that reached up to 30% of applied radioactivity.

Thus, after 32 days, most of the radioactivity in the soil was in a non-extractable form either chemically linked to humin or strongly adsorbed to inorganic or other organic soil material and can be regarded as non-mobile. Additionally, it is remarkable that the amount of non-extractable residues of the ozone samples behaved significantly ( $P < 0.05$ ) different from the control and was up to 5% higher after 8 and 32 days. A reverse effect was observed for the  $\text{CO}_2$  release (Fig. 2).

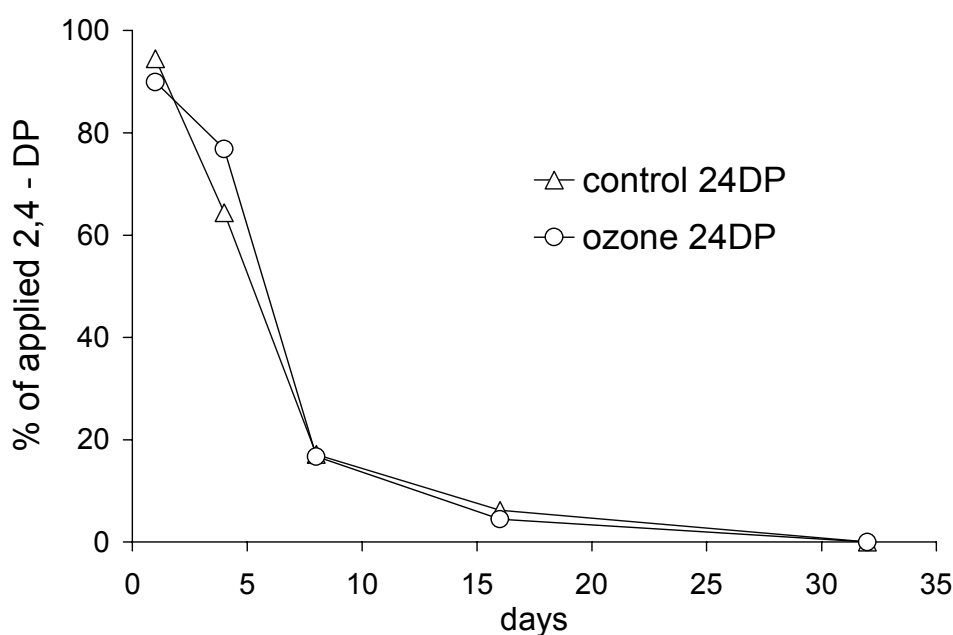


FIG 1. Percent of applied dichlorprop versus time in an aerobic degradation experiment (modified from [48]).

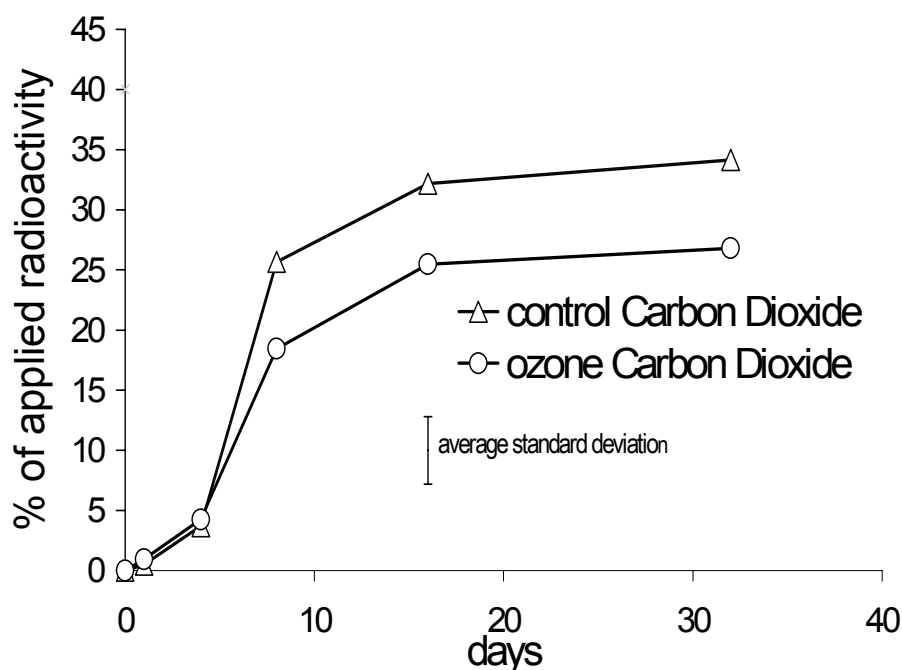


FIG 2. Carbon dioxide production versus time in an aerobic degradation experiment with dichlorprop (modified from Ref. [48]).

After 32 days mineralization of dichlorprop, CO<sub>2</sub> was up to 7% higher for the control than for the ozone treatment. This indicates an inhibition of mineralization through the presence of ozone. These suggestions are further supported by the increased C fixation to the non-extractable fraction of the ozone variant. These results suggest that, even if elevated, ozone concentrations have no influence on degradation rate and metabolism of dichlorprop in soil; mineralization and formation of non-extractable residues might be affected by the presence of increased ozone concentrations.

## 6.2. Origin of nitrate in soil water

The global N cycle is increasingly affected by industrialization. Today more atmospheric N<sub>2</sub> is converted into biologically reactive forms by anthropogenic activities than by natural processes [53]. Deposition of N-compounds may have affect N-dynamics in forest ecosystems, which are characterized by efficient recycling of nutrients, which may result in an increased leaching of nitrate into groundwater [54]. Turnover, transport, and accumulation of N in soil influences the natural abundance of the two stable isotopes <sup>14</sup>N and <sup>15</sup>N. Thus, the measurement of N-isotope composition in various compartments of the forest ecosystem might contribute considerably to understanding and even quantification of the processes and pools involved. However, due to the complexity of the chemistry of N, it might not be sufficient to rely on the N isotopes alone. In the case of nitrate, the additional measurement of <sup>18</sup>O is possible, to significantly improve the discrimination between different N-source pools. The use of <sup>15</sup>N-enriched compounds allows the direct investigation of a single soil N-pool and its distribution in other compartments [53].

Stable-isotope analysis of natural abundances of <sup>15</sup>N<sup>18</sup>O<sub>3</sub><sup>-</sup>, in combination with a <sup>15</sup>NO<sub>3</sub>-tracer study, was used to investigate the nitrate dynamics and potential groundwater pollution in a forest stand in the western area of the northern fringe of the Alps at Mühleggerköpfl in the Austrian Tyrol (900 m above sea level). The following samples (with the exception of the lysimeter leachate samples) were examined for natural isotopic variations, at intervals of 2 weeks to 2 months (with the exception of the solid samples) over a 2-year period: solid samples from four soil profiles, soil water from various depths, surface runoff, melted snow (snow lysimeter), rainfall, throughfall (i.e. rain that has passed through the canopy), mist, and leachate from the lysimeter. On June 23<sup>rd</sup>, 1998, <sup>15</sup>NO<sub>3</sub> was applied to a

monolithic lysimeter, which was established in situ (0.5 m<sup>2</sup>, 0.65 m depth). We used a high enrichment of <sup>15</sup>N (58.2%) in KNO<sub>3</sub> in order to minimize the impact of the nitrate application on the natural N dynamics of the soil: 201mg KNO<sub>3</sub> equivalent to 0.5 kg N ha<sup>-1</sup>. The measurement of isotope abundance was performed in an IRMS (Finnigan MAT 251) coupled to an elemental analyser. The results were reported in  $\delta$ -notation relative to air:

$$\delta^{15}\text{N}(\text{‰}) = 1000 \times \left( \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right)$$

where

$R_{\text{sample}}$  is <sup>15</sup>N/<sup>14</sup>N-ratio of the sample,  
and  $R_{\text{standard}}$  is <sup>15</sup>N/<sup>14</sup>N-ratio of air.

To completely isolate NO<sub>3</sub><sup>-</sup> from other O-containing compounds in the soil solution, a new sample-preparation method was developed [55]. Oxygen in NO<sub>3</sub><sup>-</sup> is converted to CO<sub>2</sub> and finally measured by the Finnigan MAT 251. The IAEA standard, Vienna Mean Ocean Water (VSMOW), was used. The contribution of NO<sub>3</sub><sup>-</sup> from the throughfall to nitrate in surface water was calculated according to the following formula (modified from Ref. [56]):

$$\% = \left( 1 - \frac{(\delta_{\text{SW}} - \delta_{\text{TF}})}{(\delta_{\text{NC}} - \delta_{\text{TF}})} \right) \times 100 \quad (3)$$

where

$\delta_{\text{SW}}$  is the  $\delta^{18}\text{O}$  value of the surface runoff water (‰),  
 $\delta_{\text{TF}}$  is the  $\delta^{18}\text{O}$  value of the throughfall (‰),  
and  $\delta_{\text{NC}}$  is the reference value of  $\delta^{18}\text{O}$  in NO<sub>3</sub><sup>-</sup> originating from nitrification processes in soil (a constant value of 12.5‰ was assumed) [57,58,59].

The horizons of the four soil profiles at Mühleggerköpfl showed  $\delta^{15}\text{N}$  values ranging between -1.4 and +1.5‰, typically increasing with depth. High variations in  $\delta^{15}\text{N}$ -NO<sub>3</sub><sup>-</sup> values of the rainfall (-5 to +6‰) indicate that NO<sub>3</sub><sup>-</sup> of different sources is deposited at that site. Mist samples were on average isotopically heavier than rainfall samples (-3 to +10.8‰). Thus, the <sup>15</sup>N abundance decreased from mist to throughfall samples. On average, we observed a higher <sup>15</sup>N abundance in precipitation samples in winter months in comparison with the summer months.

A significant correlation between the  $\delta^{15}\text{N}$ -NO<sub>3</sub><sup>-</sup> values of the surface water and soil water was obtained (Fig. 3), whereas no significant correlation between the  $\delta^{15}\text{N}$ -NO<sub>3</sub><sup>-</sup> values of any precipitation sample with the surface water could be found. Values of  $\delta^{15}\text{N}$ -NO<sub>3</sub><sup>-</sup> values in soil water and surface water exhibited typical time trends: increasing <sup>15</sup>N abundance values in spring and summer. This may be due to denitrification and turnover processes in the litter layers. In soil water,  $\delta^{15}\text{N}$ -NO<sub>3</sub><sup>-</sup> values increased with depth. This is connected with nitrification processes in various soil layers, which differ in their isotopic composition, and, additionally, with denitrification processes of nitrate originating from surface water. Due to the similarities in N-abundances of surface and soil waters, it may be concluded that nitrification processes in the litter layer are the major source for nitrate in surface water rather than nitrate deposited from the atmosphere. These conclusions are strongly supported by the  $\delta^{18}\text{O}$ -values of nitrate in various compartments. Throughfall was above 60‰, whereas NO<sub>3</sub><sup>-</sup> in surface water exhibited values between 10 and 20‰. Using formula (3), it was possible to estimate the contribution of deposited NO<sub>3</sub><sup>-</sup> to that determined in surface water. The respective portion was between 0 and 15% for different periods, with a mean value of 10%. Due to the fact that the observed  $\delta^{18}\text{O}$  values in NO<sub>3</sub><sup>-</sup> reflect the impact of manifold processes, the estimated value of a 10% contribution of deposited NO<sub>3</sub><sup>-</sup> to the surface-water NO<sub>3</sub><sup>-</sup> load has to be used with caution.

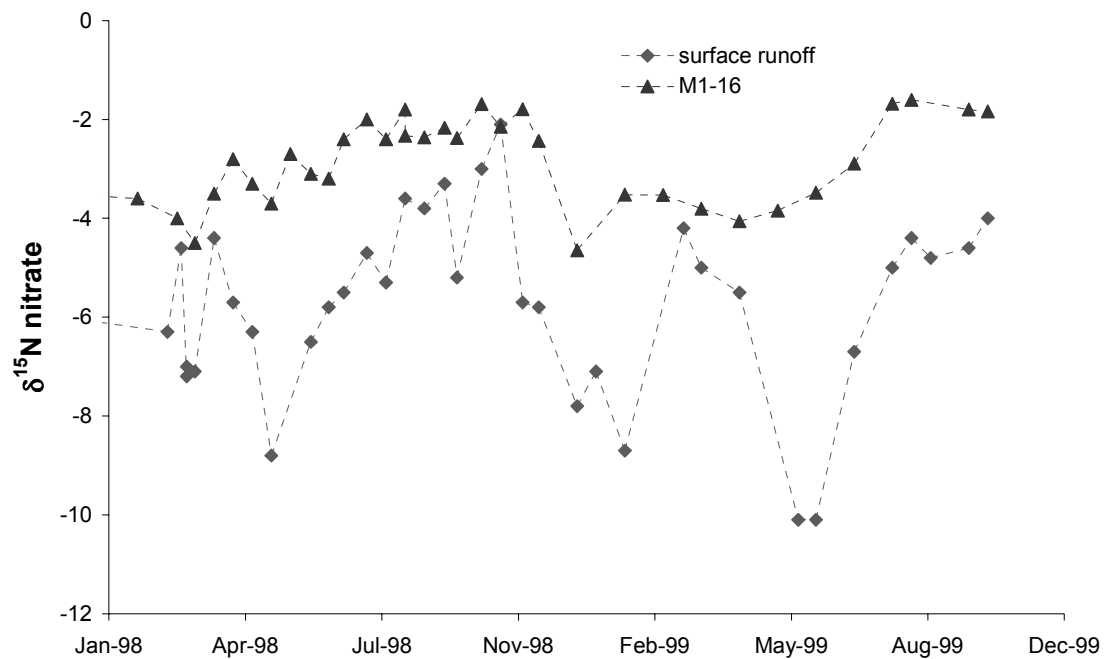


FIG. 3.  $\delta^{15}\text{N}$ -values (‰) of nitrate in surface water and soil water samples (averaged over depth, suction cups M1–16).

The main results of the lysimeter experiment are presented in Fig. 4. Two weeks after the application of  $^{15}\text{NO}_3^-$ , 1.3% of the tracer was detected in the leachate. Over the following 2 weeks, the  $\text{NO}_3^-$  leaching reached a maximum. After 130 days (November 1998) and collection of 300 L of leachate, a total of 52% of the applied  $\text{NO}_3^-$  had passed through. The rest remained in the profile due to adsorption, immobilization, and biochemical reactions. Combining the results of the tracer experiment and the investigations of the natural abundances of N and O isotopes, it was possible to estimate the contribution of deposited  $\text{NO}_3^-$  in seepage water. Five to 10% in surface water originated from deposition, 50% of which will reach deeper soil layers. Thus, a contribution of deposited  $\text{NO}_3^-$  to that in soil water at deeper horizons can be estimated at 5% ( $\pm 5\%$ ).

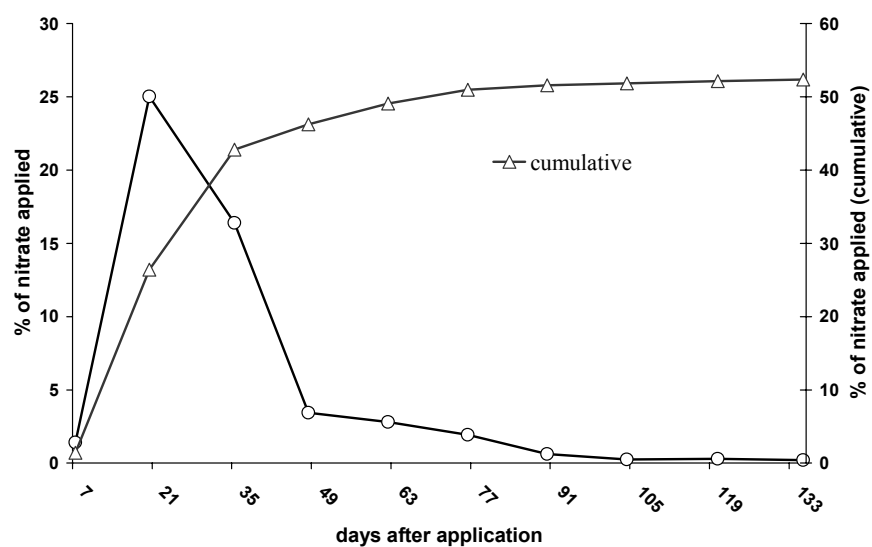


FIG. 4. Nitrate in the leachate of the monolithic lysimeter at Mühleggerköpfl in percent of the applied  $^{15}\text{N}$ -tracer.

## 7. CONCLUSIONS

The use of nuclear methods is widespread in the fields of environmental and pollution research. While some of the analytical methods, especially neutron activation analysis, have decreased in importance during recent years due to the improvement of MS-techniques, other methods, relying on X ray fluorescence or absorption, have gained significance for the study of pollution behaviour in ecosystem compartments. Isotope-dilution and tracer experiments are applied in many research fields, e.g. soil science, plant physiology, and ecology. The trend continues towards:

- combining isotopic techniques with complementary physical and chemical fractionation procedures,
- the use of lysimeters and other model systems, and
- multi-isotope approaches appropriate for addressing complicated research questions.

## REFERENCES

- [1] KOCH, H., SCHÖBER, A., Anwendung radioaktiver und stabiler Nuklide im Umweltschutz, *Isotopenpraxis* **17** (1981) 229–240.
- [2] MICHAELIS, W., Radionuclides in environmental research and protection, *Isotopenpraxis* **22** (1986) 337–344.
- [3] MAENHAUT, W., Trace element analysis of environmental samples by nuclear analytical techniques, *Int. J. PIXE* **2** (1992) 609–635.
- [4] THUN, R., et al., Die Untersuchung von Böden, 4th Edition, VDLUFA-Verlag, Darmstadt (1991).
- [5] SENGUTTA, U., Adsorption von Blei und Cadmium an Tonen—Analytische Untersuchungen. *Z. Umweltchem, Ökotox.* **5** (1993) 72–76.
- [6] STEINNES, E., Neutron activation techniques in environmental studies, *J. Radioanalytical Nuclear Chem.* **243** (2000) 235–239.
- [7] SCHNIER, C., SCHNUG, E., Spurenelementbestimmung in Pflanzenmaterial mit Instrumenteller Neutronenaktivierungsanalyse (INAA) unter besonderer Berücksichtigung des Molybdäns, *Landwirtschaftliche Forschung Sonderheft* **38** (1981) 736–750.
- [8] SPARKS, D., “Advances in understanding the kinetics and mechanisms of metal sorption at the mineral/water interface”, *Int. Symp. on Soil Mineral-Organic Matter-Microorganism Interactions and Ecosystem Health*, University of Naples, Naples (2000) 34.
- [9] SCHEINOST, A.C., et al., “Carbon-edge X ray spectromicroscopy of the colloidal fraction of two Long Island topsoils”, *Int. Symp. on Soil Mineral-Organic Matter-Microorganism Interactions and Ecosystem Health*, University of Naples, Naples (2000) 72.
- [10] TILLER, K.G., et al., Soil zinc and its uptake by plants. I. Isotopic exchange equilibria and the application of tracer techniques, *Aust. J. Soil Res.* **10** (1972) 151–164.
- [11] SINAJ, S., et al., Assessment of isotopically exchangeable zinc in polluted and nonpolluted soils, *Soil Sci. Soc. Am. J.* **63** (1999) 1618–1625.
- [12] SMOLDERS, E., et al., Cadmium fixation in soils measured by isotopic dilution, *Soil Sci. Soc. Am. J.* **63** (1999) 78–85.
- [13] SCHUG, B., et al., Improved cadmium sorption isotherms by the determination of initial contents using the radioisotope  $^{109}\text{Cd}$ , *J. Plant Nutr. Soil Sci.* **163** (1999) 197–202.
- [14] BEITZ, H., Aktuelle Aspekte der ökologisch-chemischen Bewertung von Pflanzenschutzmitteln, *Z. f. Chemie* **29** (1989) 269–276.
- [15] FÜHR, F., et al., Modification of definition of bound residues, *Pesticide Bound Residues in Soil*, Wiley-VCH, Bonn (1996) 175–176.
- [16] GEVAO, B., et al., Bound pesticide residues in soils: a review, *Environ. Poll.* **108** (2000) 3–14.
- [17] BURAUDEL, P., FÜHR, F., Formation and long-term fate of non-extractable residues in outdoor lysimeter studies, *Environ. Poll.* **108** (2000) 45–52.
- [18] KÄSTNER, M., et al., Formation of bound residues during microbial degradation of [ $^{14}\text{C}$ ]-anthracene in soil, *Appl. Environ. Microbiol.* **65** (1999) 1834–1842.
- [19] RICHNOW, H.H., et al., Tracing the transformation of labelled [ $1\text{-}^{13}\text{C}$ ]phenanthrene in a soil bioreactor, *Environ. Poll.* **108** (2000) 91–101.



- [20] BENOIT, P., PRESTON, C.M., Transformation and binding of  $^{13}\text{C}$  and  $^{14}\text{C}$ -labelled atrazine in relation to straw decomposition in soil, *Europ. J. Soil Sci.* **51** (2000) 43–54.
- [21] HAIDER, K.M., et al., “Silylation of soil organic matter – Extraction of humic compounds and soil-bound residues”, *Soil Biochemistry*, Volume 10 (BOLLAG, J.-M., STOTZKY, G., Eds.), Marcel Dekker, New York (2000) 139–170.
- [22] ARAUS, J.L., et al., Relationships between ash content, carbon isotope discrimination and yield in durum wheat, *Aust. J. Plant Physiol.* **25** (1998) 835–842.
- [23] GIARDI, M.T., et al., Effects of abiotic stresses on the turnover of the D-1 reaction centre II protein (review), *Physiol. Plantarum* **101** (1998) 635–642.
- [24] PAUSCH, R.C., et al., Use of C-13 and N-15 isotopes to investigate  $\text{O}_3$  effects on C and N metabolism in soybeans. 1. C fixation and translocation, *Agr. Ecosyst. Environ.* **59** (1996) 69–80.
- [25] McCRADY, J.K., ANDERSEN, C.P., The effect of ozone on below-ground carbon allocation in wheat, *Environ. Poll.* **107** (2000) 465–472.
- [26] SHINONAGA, T., et al., Transfer of iodine from soil to cereal grain in agricultural areas of Austria, *Sci. Tot. Environ.* **48** (1986) 33–43.
- [27] MURAMATSU, Y., OHMOMO, Y., Iodine-129 and iodine-127 in environmental samples collected from Tokaimura/Ibaraki, Japan, *Sci. Tot. Environ.* **48** (1986) 33–43.
- [28] SHINONAGA, T., et al., Determination of iodine in cereal grain and standard reference materials by neutron activation analysis, *Int J. Environ. Anal. Chem.* **78** (2000) 175–184.
- [29] GERZABEK, M.H., et al., Iodine and bromine contents of some Austrian soils and relations to soil characteristics, *J. Plant Nutr. Soil Sci.*, **162** (1999) 415–419.
- [30] FRISSEL, M.L., VAN BERGEIJK, K.E., “Mean transfer values derived by simple statistical regression analysis”, Sixth Report of IUR Working Group on Soil-to-Plant Transfer Factors, RIVM, Bilthoven (1989).
- [31] NG, Y.C., A review of transfer factors for assessing the dose from radionuclides in agricultural products, *Nucl Safety* **23** (1982) 57–71.
- [32] ROBENS, E., et al., Iodine-129 in the environment of a nuclear fuel reprocessing plant: III. Soil-to-plant concentration factors for Iodine-129 and Iodine-127 and their transfer factors to milk, eggs and pork, *J. Environ. Radioactiv.* **8** (1988) 37–52.
- [33] MURAMATSU, Y., et al., Trace experiments on transfer of radio-iodine in the soil-rice plant system, *Water Air Soil Pollut.* **45** (1989) 157–171.
- [34] AUBERT, H., PINTA, M., Trace Elements in Soils, Elsevier Scientific Publishing Company, Amsterdam (1977).
- [35] WHITEHEAD, DC., The distribution and transformations of iodine in the environment, *Environ. Int.* **10** (1984) 321–339.
- [35a] INTERNATIONAL ATOMIC ENERGY AGENCY, Handbook of Parameter Values for the Prediction of Radionuclide Transfer in Temperate Environments, IAEA Technical Reports Series 364, Vienna (1994).
- [36] YOSHIDA, S., et al., Studies on the sorption of  $\text{I}^-$  (iodide) and  $\text{IO}_3^-$  (iodate) onto Andosols, *Water Air Soil Pollut.* **63** (1992) 321–329.
- [37] MURAMATSU, Y., et al., Iodine desorption from rice paddy soil, *Water Air Soil Pollut.* **86** (1994) 359–371.
- [38] GERZABEK, M.H., et al.,  $^{137}\text{Cs}$  in soil texture fractions and its impact on  $^{137}\text{Cs}$  soil-to-plant transfer, *Comm. Soil Sci. Plant Anal.* **23** (1992) 321–330.
- [39] ARTHUR, W.J., ALLDREDGE, A.W., Importance of plutonium contamination on vegetation surfaces at Rocky Flats, CO, *Environ. Exper. Bot.* **22** (1982) 33–38.
- [40] KIRIYAMA, T., KURODA, R., Ion exchange separation and spectrophotometric determination of titanium in biological materials, *Fresenius Z. Anal. Chem.* **313** (1982) 328–330.
- [41] PINDER, J.E., McLEOD, K.W., Mass loading of soil particles on plant surfaces, *Health Physics* **57** (1989) 935–942.
- [42] LI, G., et al., An experimental study on mass loading of soil particles on plant surfaces, *Die Bodenkultur* **45** (1994) 15–24.

- [43] DREICER, M., et al., Rainsplash as a mechanism for soil contamination of plant surfaces, *Health Physics* **46** (1984) 177–187.
- [44] McLEOD, K.W., et al., Plutonium contents of broadleaf vegetable crops grown near a nuclear fuel chemical separation facility, *Health Physics* **47** (1984) 261–267.
- [45] McLEOD, K.W., et al., Contribution of a nuclear fuel chemical separations facility to the plutonium content of a tobacco crop, *Health Physics* **46** (1984) 1205–1211.
- [46] PINDER, J.E., McLEOD, K.W., Contaminant transport in agroecosystems through retention of soil particles on plant surfaces, *J. Environ. Qual.* **17** (1988) 397–407.
- [47] HAPPEMAN, C.J., et al., Total mineralization of aqueous atrazine in the presence of ammonium nitrate using ozone and *Klebsiella terrigena*, *J. Agric. Food Chem.* **43** (1995) 1383–1391.
- [48] HABERHAUER, G., et al., Effects of elevated ozone concentration on the degradation of dichlorprop in soil, *Chemosphere* **39/9** (1999) 1459–1466.
- [49] HAIDER, K., *Biochemie des Bodens*, Ferdinand Enke Verlag, Stuttgart (1995).
- [50] SMITH, A. E., Degradation, fate and persistence of phenoxyalkanoic acid herbicides in soil, *Rev. Weed Sci.*, **4** (1989) 1–24.
- [51] MÜLLER, M.D., BUSER, H.-R., Conversion reactions of various phenoxyalkanoic acid herbicides in soil, *Environ. Sci. Technol.* **31** (1997) 1953–1959.
- [52] GARRISON, A.W., et al., Enantiomeric selectivity in the environmental degradation of dichlorprop as determined by high-performance capillary electrophoresis, *Environ. Sci. Technol.*, **30** (1996) 2449–2455.
- [53] NADELHOFFER, K.J, et al., Nitrogen deposition makes a minor contribution to carbon sequestration in temperate forests, *Nature* **398** (1999) 145–148.
- [54] EMMETT, B.A., et al., Natural abundance of  $^{15}\text{N}$  in forests across a nitrogen deposition gradient, *Forest Ecol. Management* **101** (1998) 9–18.
- [55] HABERHAUER, G., BLOCHBERGER, K., A simple clean up method for the isolation of nitrate from natural water samples for O isotope analysis, *Anal. Chem.* **71** (1999) 3587–3590.
- [56] PUGET, P., et al., Total and young organic matter distributions in aggregates of cultivated soils, *Eur. J. Soil Sci.* **46** (1995) 449–459.
- [57] MAYER, B., BOLLWERK, S.M., Controls of oxygen isotope ratios of nitrate formed during nitrification in soils, IAEA-Report CSP-2, IAEA-SM-361, Vienna (2000).
- [58] GRIFFITHS, H., *Stable Isotopes – integration of biological, ecological and geochemical processes*, Bios Scientific Publishers Limited, Oxford (1998).
- [59] WASSENAAR, L.I., Evaluation of the origin and fate of nitrate in the Abbotsford aquifer using the isotopes of  $^{15}\text{N}$  and  $^{18}\text{O}$  in  $\text{NO}_3^-$ , *Appl. Geochem.* **10** (1995) 391–405.

# **RADIOECOLOGICAL ASSESSMENT OF ENVIRONMENTAL POLLUTION BY NATURALLY OCCURRING RADIONUCLIDES IN THE REGION OF AN EX-URANIUM MINE AND PLANT**

R.M. KAMENOVA-TOTZEVA, N.I. SHOPOV, R.B. KARAIVANNOVA  
National Center of Radiobiology and Radiation Protection,  
Sofia, Bulgaria

## **Abstract**

Samples of water, soil, and river sediment were collected from around the village of Elechnitzka, where a uranium mine was operational from 1946 to 1994. It was shown that levels of natural uranium, total beta activity, and radioactivity levels from radium-226, thorium-232, and potassium-40, were generally not significantly greater than background averages in Bulgarian cities. The radiation situation around the uranium milling plant was similar to those of other industrial centres and does not constitute a danger to human health.

## **1. INTRODUCTION**

Uranium was mined in the Republic of Bulgaria from 1946 to 1994. In this paper we present an assessment of the main radioecological factors around the village of Elechnitza subsequent to the closure of a nearby uranium mine. Elechnitza, a village of 3,000 inhabitants, is situated in a mountainous area of the southwest of Bulgaria, 120 km south of Sofia. There are disused mines, pits, and a milling plant, as well as a tailing pile, close to the village. The Zlataritzka River flows through Elechnitza. Before mining was curtailed, underground mine waters emptied into the river.

The presence of numerous small rocks, low in uranium content, is characteristic of the soil of the Elechnitza area. Many waste-piles are situated near the village as a result of prospecting, mining, transporting, and processing the ore.

The tailing pile is situated east of Elechnitza, in Valcho gully. Surface waters from Oreovsko and Dinderichko gullies collect as a result of drainage and flow under the tailing pile. The quantity of the deposited waste ore has been estimated at 8 Mt, with 1,600 m<sup>3</sup> of resins. The water draining from the tailing pile flows directly in the Mesta River, and does not pass through Elechnitza.

Radon and daughter products constitute the chief radiation concern, with health implications for the local population; relatively high concentrations were determined in the Elechnitza area, probably due to emanation from the waste-piles and shallow-ore materials. As a result of the radiation exposure during the mining period, and prognosis evaluations since the closure of operations, Elechnitza is considered to be at high radiation risk. The average effective annual dosage is estimated conservatively at approximately 10 to 15 mSv, whereas the average background exposure of the Bulgarian population is estimated at 2.3 mSv/year.

## **2. MATERIALS AND METHODS**

The sampling was done in the Elechnitza area after the closure of uranium mining in order to estimate the environmental radiation status. Water, soil, and sediment samples were collected. Depending on their location in relation to the industrial site and the village, the surface waters are classified in eleven groups (Table I).

The soil and sediment samples are classified as follows:

- soil from gardens and fields,
- soil from the village,
- sediment from Zlataritzka River,
- soil near the tailing pile.

The radiochemical methods used in this study are in compliance with the Bulgarian State Standards as follows:

- naturally occurring uranium, by the luminescent method, error  $\pm 20\%$ , MDA =  $11.0 \times 10^{-8}$  gU/L;
- radium-226, by measuring radon-222 activity, error  $\pm 20\%$ , MDA = 0.003 Bq/L;
- total beta activity, by beta counting of dry residue activity, error  $\pm 5\%$ .

The soils and sediments were analyzed by gamma spectrometry according to International Standard IEC 1452.

Waters of the Mesta River, Zlataritzha River, Gensko and Valcho gullies are officially classified as second-class surface flowing waters and they should correspond to the following maximum permissible amounts (MPAs):

- for naturally occurring uranium, 0.6 mg/L;
- for radium-226, 0.15 Bq/L;
- for total beta activity, 0.75 Bq/L.

According to Regulation No. 1, 1999, for radiation protection at closure of uranium mining in Bulgaria:

- for radium-226 in soils used without limitation, 200 Bq/kg;
- for radium-226 in surface water, 0.5 Bq/L;
- for naturally occurring uranium in surface water, 0.296 Bq/L;
- for total beta activity in surface water, 2.0 Bq/L.

### 3. RESULTS AND DISCUSSION

Average results for natural radionuclides in water samples, 1994 to 1999, are shown in Table I. Uranium concentrations varied between 0.0008 and 0.40 mg/L, below MPA according to Bulgarian State Standards. The specific activity of radium-226 varied from  $<0.003$  to 0.511 Bq/L. All surface-water samples, except for those from the tailing pile, met the requirements of Regulation No. 1. Only the concentration of radium-226 in Valcho gully and tailing-pile water samples exceeded MPA. The results obtained for total beta activity were from 0.098 to 1.3 Bq/L and correspond to the requirements of Regulation No. 1. Only Gensko gully water samples exceeded the MPA.

The results from analyses of soils and sediments are shown in Table II. The natural uranium concentration in agricultural soils (groups 1 and 2) varied between 11.2 and 47 mg/kg. Uranium concentrations in sediments from the Zlataritzha River were in the same range and those in soils and sediments near the tailing pile were in the range 24 to 248 mg/kg. The specific activity of radium-226 in all of the soil samples was between 60 and 700 Bq/kg. All soils except those near the contra wall of the tailing pile could be used without any limitations according to Regulation No. 1. There was slight local contamination by natural radionuclides in soils near the approach to the plant, as well as in the soil between the Zlataritzha River and Gensko gully. In comparison with the regions without additional uranium contamination, our data were three- to five-fold higher. Similar comparisons of vegetables and animal products from another regions of Bulgaria have not been made.

#### 3.1. Health status of the population

Health data collected during the period of the investigation (1997) showed no significant differences from the similar data for the control group of inhabitants near Elechnitza. There were no correlations between oncological disease or child mortality with environment parameters.

### 4. CONCLUSIONS

There is no significant surface contamination in the area around Elechnitza. The short wastewater channel flows into the Mesta River. Pure water flows into the specially constructed deviation tunnel under the tilling pile without being contaminated. Draining waters are not used for irrigation.

TABLE I. AVERAGE CONCENTRATIONS OF NATURAL RADIONUCLIDES IN WATER SAMPLES

Group	Description	Natural uranium (mg/L)	Radium-226 (Bq/L)	Total beta activity (Bq/L)
1	Natural water, not affected by mining activity	0.056 (0.035–0.062) <sup>a</sup>	0.112 (0.09–0.114)	0.55 (0.34–0.62)
2	Water from the tailing pile	0.213 (0.299–0.205)	0.511 (0.48–0.52)	0.82 (0.75–0.92)
3	Underground water from the tailing pile	0.400 (0.35–0.58)	0.390 (0.25–0.42)	0.58 (0.45–0.60)
4	Draining water from the tailing pile, running into Valcho gully	0.034 (0.026–0.038)	0.044 (0.037–0.049)	0.50 (0.48–0.52)
5	From the exit of the collector of tailing pile	0.225 (0.197–0.240)	0.103 (0.09–0.108)	0.74 (0.70–0.80)
6	100 m from the collectors exit, in Valcho gully	0.225 (0.200–0.237)	0.367 (0.34–0.38)	0.51 (0.45–0.55)
7	From Gensko gully	0.001 (0.0009–0.0015)	0.018 (0.015–0.02)	1.30 (1.12–1.40)
8	From Zlataritzia River, after settlement	0.06 (0.04–0.065)	0.01 (0.009–0.015)	0.27 (0.24–0.29)
	From river Zlataritzia River, after the mouth of Gensko gully	0.001 (0.0009–0.0015)	0.006 (0.005–0.007)	0.26 (0.22–0.28)
	From Zlataritzia River, before flowing into the Mesta River	0.067 (0.059–0.070)	0.135 (0.125–0.14)	0.75 (0.650–0.78)
9	From Mesta River, before the Zlataritzia River and Valcho gully flow into it	0.016 (0.012–0.018)	0.058 (0.046–0.064)	0.17 (0.15–0.19)
	From Mesta River, after the Zlataritzia River and Valcho gully flow into it	0.013 (0.009–0.015)	0.057 (0.045–0.063)	0.21 (0.19–0.23)
10	Water flowing from ex-shaft 54	0.0008 (0.0006–0.0009)	0.007 (0.005–0.009)	0.098 (0.075–0.15)
11	Drinking water from central water supply	0.001	0.003	0.17
	Mineral water	0.0008	0.008	0.192

<sup>a</sup>Minimum–maximum values.

Surface waters in the region are within the Quality Standards for second class waters. Only the content of the radium-226 in Valcho gully and total beta activity in Gensko gully exceeded standard values.

The radiation situation around this uranium mining plant is almost the same as in other industrial centres and does not constitute a danger to human health. However, more-sensitive methods of investigating radiation risk are recommended.

TABLE II. AVERAGE CONCENTRATIONS OF NATURAL RADIONUCLIDES IN SOILS AND SEDIMENTS

Group	Description	Natural uranium (mg/kg)	Radium-226 (Bq/kg)	Thorium-232 (Bq/kg)	Potassium-40 (Bq/kg)
1	Soils from a field, between the Zlataritzza River and Gensko gully	42 (38–44) <sup>a</sup>	239 (225–245)	47 (45–50)	840 (830–850)
	Soils from gardens under sorption columns	11.2 (11–11.4)	81 (76–85)	50 (46–53)	840 (830–850)
	Soils from gardens near the milling plant	47 (45–49)	228 (225–232)	56 (51–57)	890 (880–900)
	Soils from gardens, near the mouth of the Zlataritzza River	19.7 (19.5–20)	93 (90–95)	79 (75–82)	830 (820–840)
	Soils from a field near ex-shaft 54	19.7 (18.9–20.2)	103 (100–105)	36 (33–38)	940 (930–950)
	Soils from village gardens	17.4 (16.9–18.0)	100 (95–103)	55 (51–57)	930 (920–940)
	Soils from vegetable gardens near a waste pile	27 (25–29)	112 (109–115)	57 (55–60)	830 (820–840)
2	Soils from the village	18.2 (17.9–18.6)	60 (58–63)	56 (53–60)	880 (870–890)
3	Soils under the wall of the tailing pile	25 (23–27)	138 (135–141)	49 (47–54)	920 (910–930)
	Soils from the right side of the contra wall of the tailing pile	248 (240–253)	700 (650–720)	64 (58–67)	790 (780–800)
	Soils from the left side of the contra wall of the tailing pile	24 (21–26)	77 (70–82)	41 (37–43)	930 (920–940)
	Sediment from Gensko gully	68 (66–70)	84 (80–88)	55 (47–58)	910 (900–920)
	Sediment from Gensko gully, 400 m down-stream from the sorption columns	55 (52–57)	347 (320–354)	71 (67–72)	900 (890–910)
4	Sediment from the Zlataritzza River	11.1 (10.9–11.3)	54 (50–58)	41 (35–43)	880 (870–890)

<sup>a</sup>Minimum–maximum values.

## IODINE-131 UPTAKE AND TRANSFER FROM SOIL TO RICE FOLLOWING FACTITIOUS CONTAMINATIONS

J. BALAMURUGAN, A.R. RAJAN

Radioisotope (Tracer) Laboratory,  
Tamil Nadu Agricultural University,  
Coimbatore, India

### Abstract

An investigation was carried out to determine iodine-131 uptake and transfer factor (TF) in pot-grown rice ('IR 20') with three soils of different texture, viz., a sandy clay loam, a sandy loam, and a sandy soil. The soils were factitiously contaminated with four levels of  $^{131}\text{I}$ : 20, 40, 60, and 80 kBq kg<sup>-1</sup> of soil. Soil texture greatly influenced biomass yield,  $^{131}\text{I}$  uptake and TF in rice. The effect of level of  $^{131}\text{I}$  was significant only in the case of grain. In the case of straw, neither the  $^{131}\text{I}$  content nor uptake were influenced by the variables. The  $^{131}\text{I}$  uptake by grain appeared to increase with the level of  $^{131}\text{I}$  contamination. The highest uptake was at the highest level of  $^{131}\text{I}$  contamination and in the sandy loam soil. The TF values in all plant parts decreased significantly with the  $^{131}\text{I}$  contamination levels and were lowest, quite interestingly, in the sandy clay loam soil for root and in the sandy soil for grain. The TFs followed the order: root > straw > grain.

### 1. INTRODUCTION

Iodine-131,  $^{90}\text{Sr}$  and  $^{137}\text{Cs}$  are considered to be by far the most serious pollutants in the general environment from fallout and as atomic wastes. Although  $^{131}\text{I}$  has a short half-life (8.04 days), it is graded as a serious pollutant because of its high yield during fission, weapons testing, and reactor accidents [1].

Iodine-131 can enter the food chain through two major pathways: through consumption of food-crop grains grown on heavily contaminated soil, and by the grass/hay-cow-milk route.

Absorption of radionuclides from soil by plants is usually quantified in terms of transfer factor (TF). The observed variability of experimentally determined TFs of radionuclides in food and fodder crops complicates the value to be deployed in the prediction of the transfer of radionuclides from soil to plants in general. Thus, rather than using a single soil-to-plant TF, a crop-specific value is needed [2].

This investigation was carried out to study the effect of four levels of  $^{131}\text{I}$  contamination of soil on the content, uptake and soil-to-crop TF of  $^{131}\text{I}$  in different parts of rice, a major food crop in India.

### 2. METHODOLOGY

We determined  $^{131}\text{I}$  uptake and TF in rice (*Oryza sativa* L. 'IR 20') in a pot experiment with three soils of differing texture, viz., a sandy clay loam (Typic Haplustert), a sandy loam (Mixed Typic Ustipsamment), and a sandy soil (Typic Ustropept).

Aliquots of 12 kg of 2-mm-sieved soil were placed in ceramic pots of diameter 30 cm and height 30 cm. The soils were brought to a puddled condition and contaminated with  $^{131}\text{I}$ , as carrier-free sodium iodide in dilute sodium thiosulphate medium, at four levels: 20, 40, 60 and 80 kBq kg<sup>-1</sup> of soil. The treatments were replicated twice in a factorial completely randomized design. Common basal applications of N (60 kg N ha<sup>-1</sup> as urea), P (26 kg P ha<sup>-1</sup> as superphosphate), and K (50 kg K ha<sup>-1</sup> as potassium chloride) were made to the pots. Rice seedlings (21 days old) were then transplanted into the pots at six per pot in three hills. A month later, a top-dressing with urea (30 kg N ha<sup>-1</sup>) was given, followed a month later by another dose of 30 kg N ha<sup>-1</sup>. The crop was harvested at maturity as root, straw, and grain. The oven dry weights of the samples were recorded. The samples were analyzed for  $^{131}\text{I}$  activity using a NaI (TI) gamma ray spectrometer. From the radioassay data, the  $^{131}\text{I}$  content, uptake, and transfer factor (TF) were computed [3].

### 3. RESULTS

#### 3.1. Biomass yield

Level of  $^{131}\text{I}$  had a significant influence only in the case of grain. As the level of  $^{131}\text{I}$  contamination increased, the biomass of the grain also increased, culminating in the highest biomass at the highest level of  $^{131}\text{I}$  (Table I). Of the three soils, the biomass yields of grain and straw were highest in the sandy loam (Table II), due to its inherent fertility. This soil, on a comparative basis, had higher CEC, was neutral in pH, and was richer in available N, P and K.

#### 3.2. Iodine-131 content

The highest  $^{131}\text{I}$  content in roots was observed in the sandy soil (Table III). This was so irrespective of the level of contamination. With its high sand content (77%) and less organic matter, this soil might have retained less  $^{131}\text{I}$  and thus rendered more for uptake. A significant negative correlations between the organic C, clay, and  $^{131}\text{I}$  content in roots supports this.

TABLE I. EFFECT OF LEVEL OF  $^{131}\text{I}$  ON THE BIOMASS YIELD OF RICE GRAIN

Level of $^{131}\text{I}$ (kBq kg <sup>-1</sup> soil)	Biomass yield (g pot <sup>-1</sup> )			Mean
	Sandy clay loam	Sandy loam	Sandy soil	
20	10.6	11.5	9.90	10.7b
40	8.45	15.4	10.3	11.4b
60	11.3	17.2	10.5	13.0ab
80	13.0	18.8	11.5	14.4a
Mean	10.8b	15.7a	10.6b	

Means within a column or row followed by the same letter are not significantly different at  $P = 0.05$ .

TABLE II. EFFECT OF LEVEL OF  $^{131}\text{I}$  ON THE BIOMASS YIELD OF RICE STRAW

Level of $^{131}\text{I}$ (kBq kg <sup>-1</sup> soil)	Biomass yield (g pot <sup>-1</sup> )			Mean
	Sandy clay loam	Sandy loam	Sandy soil	
20	13.4	12.1	10.3	12.0a
40	16.2	13.8	10.1	13.4a
60	13.2	15.0	10.5	12.9a
80	12.3	17.0	10.4	13.2a
Mean	13.8a	14.5a	10.3b	

TABLE III. EFFECT OF LEVEL OF  $^{131}\text{I}$  ON THE  $^{131}\text{I}$  CONTENT OF RICE ROOT

Level of $^{131}\text{I}$ (kBq kg <sup>-1</sup> soil)	$^{131}\text{I}$ content (kBq kg <sup>-1</sup> )			Mean
	Sandy clay loam	Sandy loam	Sandy soil	
20	752	1,760	2,428	1,647a
40	757	1,402	2,102	1,420a
60	2,153	1,710	1,972	1,945a
80	1,988	1,953	1,742	1,894a
Mean	1,413b	1,706ab	2,061a	



TABLE IV. EFFECT OF LEVEL OF  $^{131}\text{I}$  ON THE  $^{131}\text{I}$  CONTENT IN RICE GRAIN

Level of $^{131}\text{I}$ (kBq kg $^{-1}$ soil)	$^{131}\text{I}$ content (kBq kg $^{-1}$ )			Mean
	Sandy clay loam	Sandy loam	Sandy soil	
20	367	702	245	438c
40	1,007	690	757	818a
60	450	650	605	568bc
80	608	578	836	674ab
Mean	608a	655a	611a	

Means within a column or row followed by the same letter are not significantly different at  $P = 0.05$ .

TABLE V. EFFECT OF LEVEL OF  $^{131}\text{I}$  ON THE  $^{131}\text{I}$  UPTAKE BY RICE GRAIN

Level of $^{131}\text{I}$ (kBq kg $^{-1}$ soil)	$^{131}\text{I}$ uptake (kBq pot $^{-1}$ )			Mean
	Sandy clay loam	Sandy loam	Sandy soil	
20	3.89	7.98	2.43	4.77b
40	8.48	10.6	7.77	8.95a
60	5.07	11.3	6.55	7.64a
80	7.87	10.9	9.58	9.46a
Mean	6.33b	10.2a	6.58b	

Level of  $^{131}\text{I}$  contamination significantly influenced its content in rice grain (Table IV). An increasing trend was observed with increasing contamination level up to 40 kBq kg $^{-1}$ , beyond which the content decreased. This was presumably because the iodine requirement of the crop reached saturation with the 40 kBq kg $^{-1}$  level.

With regard to straw, none of the variables had any significant effect. Among the plant parts, roots recorded higher  $^{131}\text{I}$  content than the above-ground parts. This implied that the transfer from the root to the above-ground parts was not commensurate with the transfer from soil to root.

### 3.3. Iodine-131 uptake

Soil type and level of  $^{131}\text{I}$  contamination had significant effects on  $^{131}\text{I}$  uptake only with respect to grain (Table V). Just as grain biomass was influenced by the soils, so was uptake. Likewise, the  $^{131}\text{I}$  content in grain was also influenced by the level of  $^{131}\text{I}$  and soils. Hence the observed trend.

As the result of higher  $^{131}\text{I}$  content and biomass of grain than in the other two soils, the sandy loam soil recorded the highest  $^{131}\text{I}$  uptake values.

### 3.4. Soil-to-crop transfer factor

Absorption of a radionuclide from soil by a crop is quantified in terms of transfer or concentration factor, which is defined as the ratio of the radioactivity per unit dry weight of the plant (or individual organ) and the activity per unit dry weight of the soil in the root zone.

Soil-to-crop TFs are predominantly a function of soil type. This was clear in the case of rice root and grain, though not in the case of straw (Tables VI–VIII). The sandy soil gave the highest TF values in root, whereas in grain, it was the sandy loam soil. The low sorption of  $^{131}\text{I}$  in these soils, due to light texture, evidently produced relatively high TF values.

TABLE VI. EFFECT OF LEVEL OF  $^{131}\text{I}$  ON THE SOIL-TO-CROP TF OF  $^{131}\text{I}$  IN RICE ROOT

Level of $^{131}\text{I}$ (kBq kg <sup>-1</sup> of soil)	Transfer factor			Mean
	Sandy clay loam	Sandy loam	Sandy soil	
20	62.6	88.0	121	90.7a
40	31.4	35.0	52.6	39.7b
60	35.9	28.5	32.9	32.4b
80	24.9	24.4	21.8	23.7c
Mean	38.7b	44.0b	57.2a	

Means within a column or row followed by different letters are significantly different at  $P = 0.05$ .

TABLE VII. EFFECT OF LEVEL OF  $^{131}\text{I}$  ON THE SOIL-TO-CROP TF OF  $^{131}\text{I}$  IN RICE STRAW

Level of $^{131}\text{I}$ (kBq kg <sup>-1</sup> of soil)	Transfer factor			Mean
	Sandy clay loam	Sandy loam	Sandy soil	
20	50.5	48.5	47.9	49.0a
40	25.9	29.2	21.6	25.6b
60	17.2	15.3	11.3	14.6c
80	6.75	9.23	12.4	9.45c
Mean	25.1a	25.6a	23.3a	

TABLE VIII. EFFECT OF LEVEL OF  $^{131}\text{I}$  ON THE SOIL-TO-CROP TF OF  $^{131}\text{I}$  IN RICE GRAIN

Level of $^{131}\text{I}$ (kBq kg <sup>-1</sup> of soil)	Transfer factor			Mean
	Sandy clay loam	Sandy loam	Sandy soil	
20	18.3	35.1	12.2	21.9a
40	25.2	17.2	18.9	20.4a
60	7.49	10.8	10.1	9.47b
80	7.60	7.23	10.4	8.43b
Mean	14.6b	17.6a	12.9b	

The levels of  $^{131}\text{I}$  significantly influenced the TF values in individual parts of the rice plant. Level of  $^{131}\text{I}$  and TF value had an inverse relationship. Decreasing TF with increasing contamination levels in soil might be attributed to the non-linear relationships between ion-uptake by plant roots and ion concentration in the rooting medium. The TF in rice followed the following order: root > straw > grain. Lower TF values in grain, as compared to straw and roots, have been reported before [4,5].

#### 4. CONCLUSIONS

Our results show that soil texture greatly influenced biomass yield,  $^{131}\text{I}$  uptake, and TF in rice. The effect of level of  $^{131}\text{I}$  was significant only in the case of grain. For straw, neither the  $^{131}\text{I}$  content nor uptake were influenced by either of the variables. The  $^{131}\text{I}$  uptake by grain appeared to increase with the level of  $^{131}\text{I}$  contamination. The highest uptake was at the highest level of  $^{131}\text{I}$  contamination and in the sandy loam soil.

The TF values in all the three plant parts decreased significantly with increasing  $^{131}\text{I}$  contamination levels and were lowest, quite interestingly, in the sandy clay loam soil for root and in the sandy soil for grain. The TF followed the order: root > straw > grain.

## ACKNOWLEDGEMENTS

This study forms part of a project funded by the Atomic Energy Regulatory Board (AERB), Government of India. The authors thank profusely the AERB for the financial support and Dr. A.R. Sundararajan, Head, Health & Safety Division, AERB, Mumbai, for suggestions.

## REFERENCES

- [1] HOWARD, B.J., A Review of Available Countermeasures to Reduce Radioiodine Transfer to Milk-With Special Regard to the Use of Stable Iodine, Merlewood Research Station, Grange-over-sands, Cumbria (1994).
- [2] ROBENS, E.J., et al., Iodine-129 in the environment of a nuclear fuel reprocessing plant. III. Soil-to-plant concentration factors for iodine-129 and iodine-127 and their transfer factors to milk, eggs and pork, J. Environ. Radioactivity **37** (1988) 52–60.
- [3] INTERNATIONAL ATOMIC ENERGY AGENCY, Guidelines for Agricultural Countermeasures Following an Accidental Release of Radionuclides, A Joint Undertaking by the IAEA and FAO, Technical Reports Series No. 363, IAEA, Vienna (1994).
- [4] MURAMATSU, Y., et al., “Transfer of radioiodine from the environment to rice plants”, Transfer of Radionuclides in Natural and Semi-natural Environments (DESMAT, G., et al., Eds.), Elsevier Applied Science, New York (1990) 619–625.
- [5] MACKOWIAK, C.L., GROSSL, P.R., Iodate and iodine effects on iodine uptake and partitioning in rice (*Oryza sativa* L.) grown in solution culture, Plant Soil **212** (1999) 135–143.

# **ASSESSMENT OF SOIL EROSION AND SEDIMENTATION**

(Session 6)



## Keynote Address

### RECENT ADVANCES IN THE USE OF ENVIRONMENTAL RADIONUCLIDES IN SOIL EROSION INVESTIGATIONS

D.E. WALLING

Department of Geography,  
University of Exeter,  
Exeter, United Kingdom

#### Abstract

Although recent concern for the global environment has tended to highlight global warming and climate change, soil erosion and associated land degradation remain serious problems. There is an important need for reliable information, both to quantify the problem and to underpin the development of effective soil conservation strategies. Traditional approaches to documenting erosion rates are capable of meeting some needs, but they possess a number of important limitations. The use of environmental radionuclides, and more particularly  $^{137}\text{Cs}$ , has attracted increasing interest as a means of documenting rates and patterns of soil loss. The approach was first employed in the early 1970s, and since that time it has been refined and its application has become increasingly standardized. This paper reviews what are seen to be important *developments* of the  $^{137}\text{Cs}$  technique in recent years, related to this refinement and standardization. These developments have been coupled with an expansion in the application of the technique beyond the basic quantification of rates and patterns of soil loss. Two examples of *new applications*, involving the elucidation of erosion / crop productivity relationships and the validation and calibration of distributed soil erosion and soil delivery models, are described. In addition, recent years have seen an *extension* of the technique to embrace the use of other radionuclides, both as an alternative and a complement to  $^{137}\text{Cs}$ . Recent developments are discussed in the use of excess  $^{210}\text{Pb}$  and  $^7\text{Be}$  in soil erosion investigations, to document both longer and shorter timescales than those possible with  $^{137}\text{Cs}$ .

#### 1. THE CONTEXT

Although recent concern for the global environment has tended to highlight threats posed by global warming and climate change, soil erosion and associated land degradation undoubtedly remain serious problems. Population growth and the associated expansion and intensification of agricultural activity in many areas of the world have caused increased rates of soil loss and threaten the longer term sustainability of the global soil resource. It has recently been estimated that about 80% of the world's agricultural land suffers from moderate to severe erosion and that, worldwide, about  $12 \times 10^6$  ha of arable land are destroyed or abandoned annually as a result of non-sustainable farming practices [1]. On the remaining eroding land, soil loss commonly reduces productivity, as a result of reduced soil depth and water-storage capacity and the loss of nutrients. Because of this erosion-induced loss of productivity and population growth, the global per-capita food supply is currently declining [1]. In many areas of the world, these on-site impacts of increased soil loss are frequently coupled with serious off-site impacts related to the increased mobilization of sediment and its delivery to river systems [2]. These off-site impacts include water pollution, reservoir sedimentation, the degradation of aquatic habitats, and the increased cost of water treatment. Globally, the current economic cost of the on-site and off-site impacts of erosion of agricultural land has been estimated to amount to ca. \$400 billion/year [1].

Numerous other statistics could be cited to emphasize the seriousness of the soil erosion problem, but it is also important to recognize that excessive soil loss can be successfully countered by effective soil-conservation programmes. In the United States, for example, total erosion on cropland was estimated to have decreased by 42% between 1982 and 1997, as a result of removal of highly erodible cropland from production and improved conservation measures [1]. Similarly, recent reduction in the sediment load of the Yellow River in China partly reflects the extensive implementation of soil- and water-conservation measures within the highly erodible loess region of the middle Yellow River basin [3].

Current concern over on-site and off-site problems associated with accelerated soil loss is generating an increasing need for reliable estimates of erosion in various areas of the world, in order to provide a more comprehensive assessment of the magnitude of the problem and its controls, and of the link between soil loss and reduced crop productivity. Equally, the successful implementation of erosion-control strategies requires reliable information on rates of soil loss and sediment delivery to river systems, in order to establish the influence of key controlling variables to assess the effectiveness of specific control measures and to target available resources. Traditional approaches to documenting erosion rates can meet some of these needs, but such approaches possess a number of important limitations [4,5]. These include representativeness, cost, the need for long term monitoring, and operational constraints. In addition, recent advances in the use of distributed numerical models and the application of GIS and geostatistics to erosion modelling [e.g. 6–8] have highlighted the requirement for spatially distributed data capable of representing the spatial variability of erosion and deposition rates within the landscape, in response to the local topography and land use. The results provided by erosion plots, which relate to the net soil flux across the lower boundary of a small artificially isolated area, have proved invaluable for assessing the influence of various crops and land-use practices on erosion rates, but are much less able to meet the requirement for spatially distributed data. In some situations, lack of reliable direct measurements of erosion rates can be overcome by the use of prediction equations such as USLE and RUSLE [9], but independent verification is generally required and lack of confidence in such equations means that there remains an important need for direct assessment. The quest for alternative techniques for soil erosion assessment, both as an alternative and a complement to existing methods and to meet new data requirements, has directed attention to the use of environmental radionuclides, and more particularly  $^{137}\text{Cs}$ , as tracers for documenting rates and patterns of soil redistribution within the landscape. This approach has been shown to have considerable potential.

## 2. THE USE OF $^{137}\text{Cs}$ MEASUREMENTS IN SOIL EROSION INVESTIGATIONS

### 2.1. The basis

The basis of the  $^{137}\text{Cs}$  technique has been fully described [e.g. 10–12]. In essence, it exploits the fact that the worldwide fallout of radiocaesium produced by the atmospheric testing of thermonuclear weapons during the 1950s and 1960s was in most environments rapidly and strongly adsorbed by the surface soil. Subsequent redistribution of the  $^{137}\text{Cs}$  fallout will have occurred in association with the movement of soil and sediment particles within the landscape, and is thus a direct reflection of erosion and deposition mechanisms. By establishing a local reference inventory that represents the  $^{137}\text{Cs}$  inventory at a stable site, where neither erosion nor deposition has occurred, it is possible to compare the inventories measured at individual points with the reference value and to identify areas where erosion (reduced inventories) and deposition (increased inventories) have occurred. The precise magnitude of the decrease or increase in the  $^{137}\text{Cs}$  inventory at a particular point can also be used to estimate the mean erosion or deposition rate at that point for the period since the fallout input (i.e. ca. 40 years), by using a calibration procedure or model that relates the erosion or deposition rate to the magnitude of the reduction or increase in the  $^{137}\text{Cs}$  inventory, respectively [13].

The potential of the  $^{137}\text{Cs}$  technique to provide spatially distributed information on medium-term (i.e. 35–40 years) rates and patterns of soil redistribution within the landscape is usefully demonstrated by Fig. 1 and Table I, which present the results obtained from a study of parts of two small fields at Aller Barton Farm (7.5 ha) and Higher Walton Farm (6 ha) near Crediton in Devon, UK. Soil cores for subsequent  $^{137}\text{Cs}$  analysis were collected from the two fields at the intersections of a 20-m grid, providing 158 and 144 cores, respectively. Figure 1 emphasizes the spatially distributed nature of the results obtained, and Table I demonstrates the ability of the approach to provide information on gross and net erosion rates, deposition rates, and the overall sediment delivery ratio for the field. Both aspects must be seen as representing key advantages over traditional approaches to documenting soil erosion. Table II summarizes these and other key advantages of the  $^{137}\text{Cs}$  technique and particular emphasis must be placed on the ability to obtain *retrospective* information on the basis of a single site visit.

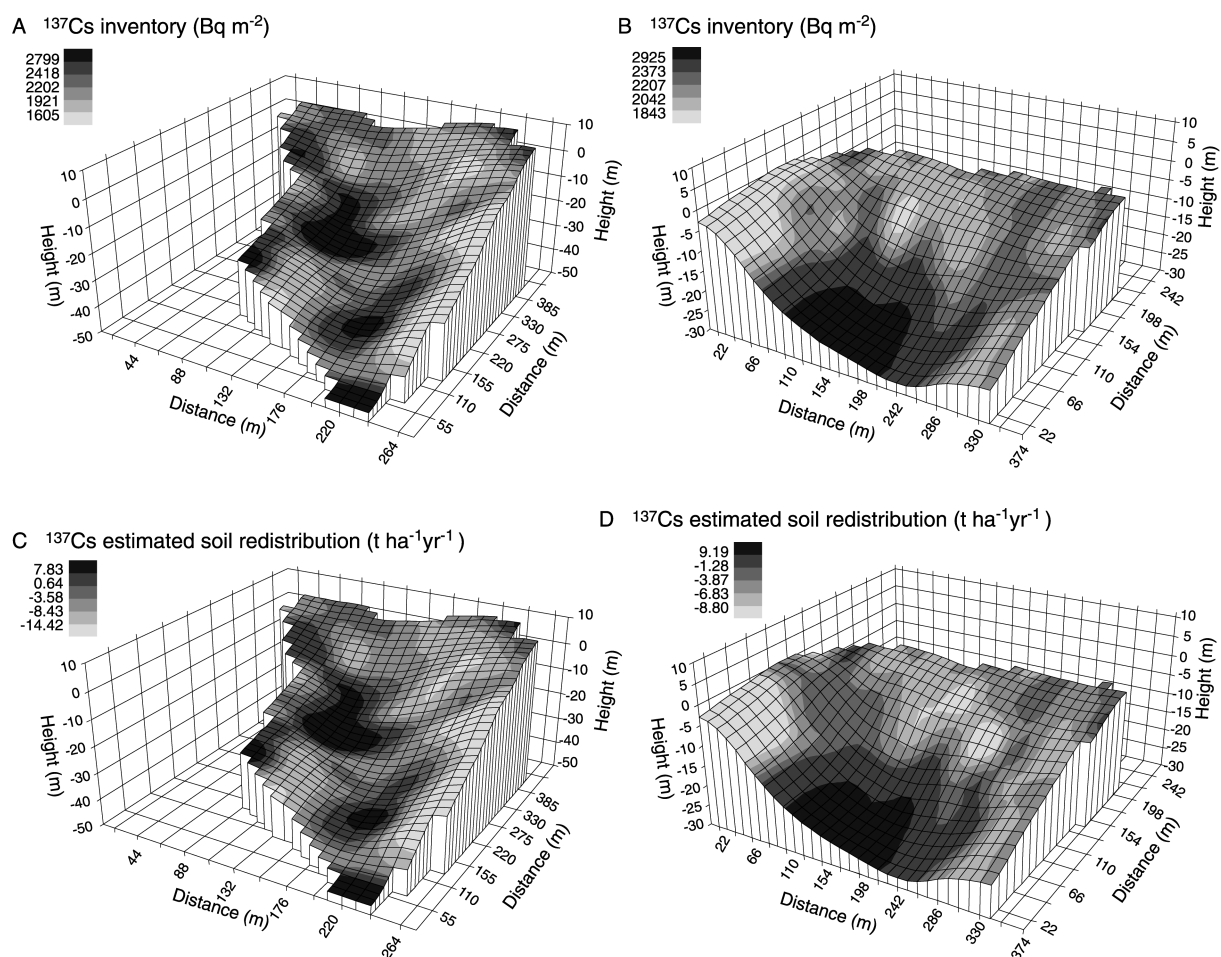


FIG. 1. The use of  $^{137}\text{Cs}$  measurements to document the magnitude of spatial distribution of erosion and deposition rates within fields at Aller Barton Farm (A, C) and Higher Walton Farm (B, D) near Crediton, Devon, UK..

TABLE I. SPATIALLY INTEGRATED ESTIMATES OF MEDIUM-TERM SOIL REDISTRIBUTION RATES IN THE TWO FIELDS ILLUSTRATED IN FIG. 1

Value estimated	Aller Barton Farm	Higher Walton Farm
Gross erosion rate ( $\text{t ha}^{-1} \text{yr}^{-1}$ )	6.8	4.4
Mean erosion rate in the eroding areas ( $\text{t ha}^{-1} \text{yr}^{-1}$ )	10.4	6.2
Mean deposition rate for the depositional areas ( $\text{t ha}^{-1} \text{yr}^{-1}$ )	14.5	7.4
Net erosion rate ( $\text{t ha}^{-1} \text{yr}^{-1}$ )	2.7	2.9
Sediment delivery ratio (%)	40	66

Although Table II emphasizes the key advantages of the  $^{137}\text{Cs}$  technique, it is necessary to recognize that the approach inevitably has some limitations. These include cost and, more particularly, the need for specialized laboratory facilities for undertaking the  $^{137}\text{Cs}$  analyses by gamma spectrometry, uncertainties associated with the calibration procedures used to estimate erosion and deposition rates from the  $^{137}\text{Cs}$  measurements, the inability to document short term changes in erosion rates, such as



those associated with changing land-use practices, and the restriction of the technique to the measurement of sheet erosion, although rill erosion can also be documented on cultivated land where the rills are regularly obliterated by cultivation. The significance of these limitations, particularly those of cost, must, however, be evaluated in light of the particular advantages of the  $^{137}\text{Cs}$  technique and the fact that all other approaches also involve important limitations.

## 2.2. The current status of the $^{137}\text{Cs}$ technique

Work in the 1960s on the use of fallout radionuclides as a tool in soil erosion investigations demonstrated the link between the post-fallout redistribution of  $^{90}\text{Sr}$  and  $^{137}\text{Cs}$  and soil erosion (cf. Refs. [15–17]). However, Ritchie and McHenry, working in the late 1960s and early 1970s, must be credited with highlighting the potential for using measurements of bomb-derived  $^{137}\text{Cs}$  as a means of estimating rates of soil loss [18–20]. Somewhat surprisingly, others were slow to exploit this development, but during the 1980s the potential for using  $^{137}\text{Cs}$  measurements in soil erosion investigations was increasingly recognized (cf. Ref. [14]). This expansion of activity continued into the 1990s, and a recent survey undertaken by the author has identified more than 120 locations at which  $^{137}\text{Cs}$  has been successfully employed for estimating erosion rates. The distribution of these locations (Fig. 2), indicates that the technique has been demonstrated to be globally applicable, despite the fact that  $^{137}\text{Cs}$  inventories are known to decline away from the mid-latitudes of the northern hemisphere, where the first studies were undertaken, and to be almost an order of magnitude less over much of the southern hemisphere. In the absence of detailed records for large areas of the globe, no detailed maps of the global distribution of  $^{137}\text{Cs}$  fallout are currently available. However, by coupling the available data on  $^{137}\text{Cs}$  fallout with information on the magnitude of bomb-derived  $^{90}\text{Sr}$  fallout, for which more records are available and to which  $^{137}\text{Cs}$  fallout is directly related, it is possible to generate an approximate map of the global pattern of  $^{137}\text{Cs}$  fallout, such as that in Fig. 3. Improvements in gamma-detector efficiency and resolution have meant that the low inventories found in many areas of the world can still be accurately measured and used as a basis for estimating soil erosion rates [21–23].

TABLE II. KEY ADVANTAGES OF THE  $^{137}\text{Cs}$  TECHNIQUE IN SOIL EROSION INVESTIGATIONS (based in part on Ref. [14])

- 
- |     |                                                                                                                                                                                                                        |
|-----|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| (1) | Estimates relate to individual points within the landscape and information relating both to rates and spatial patterns of soil redistribution can be assembled.                                                        |
| (2) | The technique is capable of providing spatially distributed data that are compatible with recent advances in physically based distributed modelling and the application of GIS and geostatistics to erosion modelling. |
| (3) | The estimated rates of soil redistribution represent the integration of all landscape processes resulting in the movement of soil particles (e.g. water and wind erosion, tillage redistribution).                     |
| (4) | Permits quantification of soil loss and deposition associated with sheet erosion, which are frequently difficult to identify in the field.                                                                             |
| (5) | The estimated rates of soil redistribution relate to the past 40 years and thus provide estimates of longer-term average rates of erosion and deposition. Short term measurements may be unrepresentative.             |
| (6) | There are no major scale constraints apart from the number of samples that can be processed. Areas studied can range from a few $\text{m}^2$ to small drainage basins (e.g. 5 ha).                                     |
| (7) | The measurements required do not necessitate significant disturbance of the landscape under study                                                                                                                      |
| (8) | Estimates can be obtained on the basis of a single site visit.                                                                                                                                                         |
| (9) | Estimates based on contemporary sampling are retrospective and, therefore, avoid the need for the establishment of long term monitoring programmes.                                                                    |
-

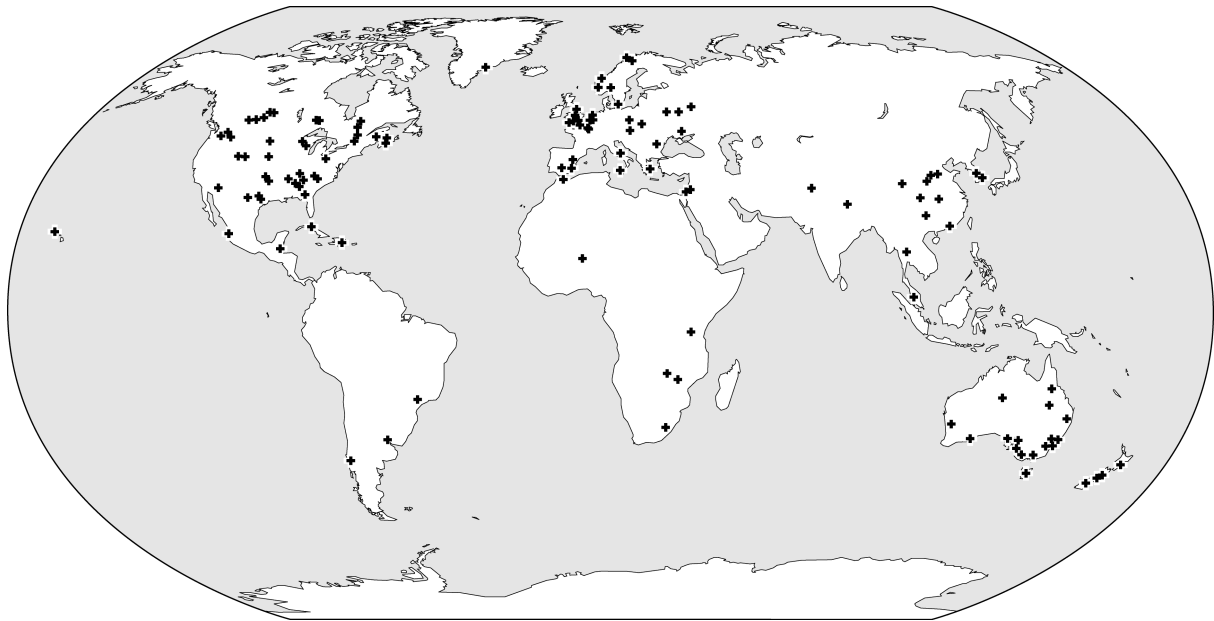


FIG. 2. Distribution of studies that have successfully employed  $^{137}\text{Cs}$  in investigations of soil erosion.

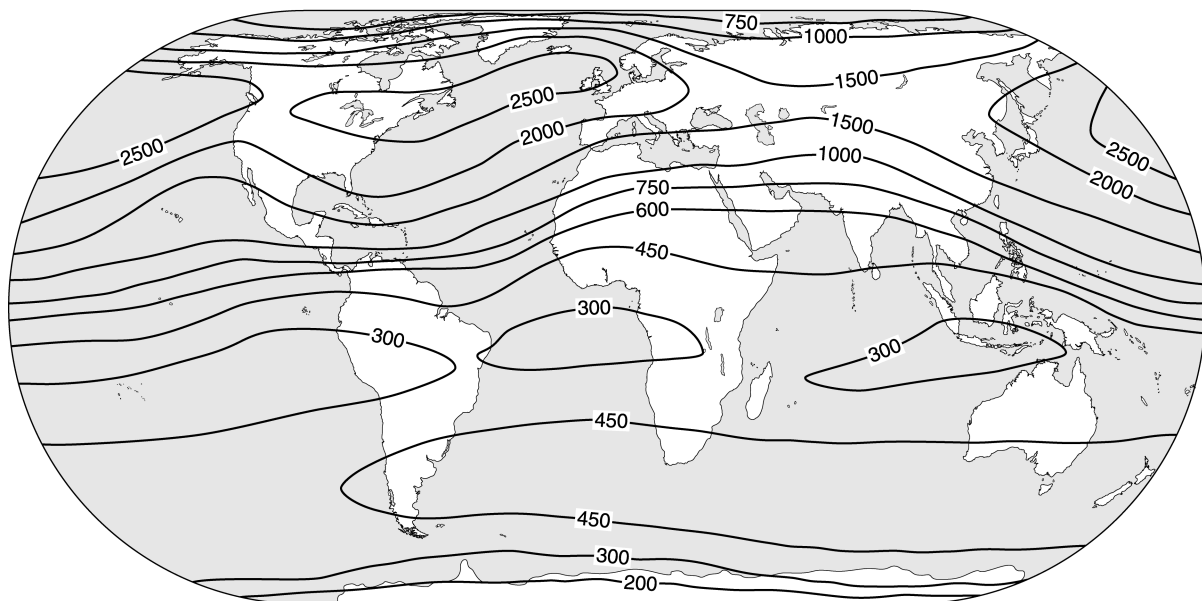


FIG. 3. An approximate representation of the global distribution of bomb-derived  $^{137}\text{Cs}$  fallout inventories in 1998 (based on information in Refs [24–26]).

### 2.3. Recent developments in the $^{137}\text{Cs}$ technique

The considerable expansion in the application of the  $^{137}\text{Cs}$  technique that occurred during the 1980s and into the 1990s was coupled with many developments. These developments addressed apparent limitations in the approach, introduced a degree of standardization into the procedures adopted, and exploited new opportunities. What are seen to be amongst the most important of these developments are briefly reviewed below.

### *2.3.1. Establishment of reference inventories*

As indicated above, a key element of the  $^{137}\text{Cs}$  technique is the need to compare the  $^{137}\text{Cs}$  inventory measured at a specific point with the local reference inventory, in order to determine the reduction or increase in the inventory, that can, in turn, be used to estimate the erosion or deposition rate. The establishment of an accurate value for the reference inventory is clearly of critical importance to the validity of the final estimate of the erosion or deposition rate. In early studies involving the  $^{137}\text{Cs}$  technique, much attention was directed to the criteria for the selection of reference sites [e.g. 27, 28]. More recently, attention has rightly turned to the variability of inventory measurements and the need to collect multiple samples, in order to define the sampling statistics associated with the value obtained for the reference inventory, and thereby take explicit account of this variability [e.g. 29–31]. Owens and Walling [32] have further proposed that this variability can be ascribed to four potential sources reflecting firstly, measurement precision, secondly, sampling variability associated with the use of a small sampling area, thirdly, random spatial variability associated with the small-scale variations in the spatial distribution of  $^{137}\text{Cs}$  resulting from heterogeneity of soil properties, microtopography and related controls, and, finally, systematic variability operating over larger areas in response to controls such as variations in precipitation amount, topography, and vegetation cover. Sampling and measurement programmes must be designed to take account of each of these potential sources of variability, and it is important to recognize that the final value used for the reference inventory should be seen as representing a range, rather than an absolute value, in order to reflect this inherent variability [e.g. 33]. Similar considerations should be applied to the values of  $^{137}\text{Cs}$  inventory obtained for individual measuring points, since these will also reflect both measurement precision and sampling variability. More is now known about the systematic variation of reference inventories across an area or region, as a result of several recent studies that attempted to identify the key controls [e.g. 34, 35].

### *2.3.2. Deriving estimates of rates of erosion and deposition from $^{137}\text{Cs}$ measurements*

Once the deviation of the  $^{137}\text{Cs}$  inventory, measured at an individual point, from the reference inventory has been established, this value must be converted to an estimate of the erosion or deposition rate associated with that point. A wide range of calibration procedures have been employed for this purpose, and a distinction can usefully be drawn between empirical relationships and theoretical models. The former are based on relationships between the loss or gain in  $^{137}\text{Cs}$  inventory relative to the local reference inventory, and independent measurements of erosion rates available for the same site, such as might be obtained from erosion plots. The latter make use of a theoretical representation of the fate of the  $^{137}\text{Cs}$  input to a soil profile, to define the relationship between the loss or gain in  $^{137}\text{Cs}$  inventory and the erosion or deposition rate. In addition, a distinction is commonly drawn between cultivated soils and undisturbed soils (e.g. permanent pasture and rangeland) in applying such calibration procedures. The relationships or models employed differ between the two cases, since in cultivated soils the  $^{137}\text{Cs}$  fallout will be mixed within the plough layer by cultivation, whereas in the case of a pasture soil the  $^{137}\text{Cs}$  will remain near the soil surface. Removal of a given depth of soil by erosion is, therefore, likely to remove a much greater proportion of the total  $^{137}\text{Cs}$  inventory from a pasture soil than from a cultivated soil.

It is clear that the choice of calibration relationship is likely to exert an important influence on the accuracy of the resulting estimates of erosion and deposition rates, and the many uncertainties associated with the relationships employed by studies prior to the late 1980s were reviewed by Walling and Quine [13]. Based on their work, Fig. 4 provides a useful indication of the nature and extent of this uncertainty by illustrating for a hypothetical site the many different relationships that have been used to estimate rates of soil loss from  $^{137}\text{Cs}$  measurements. Some of the differences between the calibration procedures evident on Fig. 4 can be accounted for by the inclusion of both cultivated and uncultivated soils, and empirical relationships derived for different environments. However, much of the spread is simply a reflection of the different assumptions and relationships employed in the various studies, and should be seen as introducing a substantial degree of uncertainty in relation to the resulting estimates of erosion rate. This uncertainty has, indeed, sometimes been cited as an important limitation of the  $^{137}\text{Cs}$  approach. The basic assumptions associated with the simple Proportional Model, which has been widely used to estimate erosion rates on cultivated soils, are, for

example, invalid. In this model, it is assumed that the depth of soil eroded during the period since the onset of bomb fallout, expressed as a proportion of the plough depth, is directly proportional to the proportion of the reference inventory that has been lost. This assumption ignores the fact that as the topsoil is eroded, the remaining  $^{137}\text{Cs}$  will be mixed into the underlying subsoil as this is progressively incorporated into the plough layer. Thus, even when the depth of soil eroded is equivalent to the total plough depth, some  $^{137}\text{Cs}$  will still remain within the plough layer. The proportional model will, therefore, underestimate the erosion rate associated with the loss of a given proportion of the reference inventory. The likely degree of underestimation is indicated in Fig. 5, which compares the relationships between soil erosion rate and percentage  $^{137}\text{Cs}$  inventory loss derived for a hypothetical soil using the proportional model and a mass-balance model. The mass-balance model takes into account the continued mixing of the remaining  $^{137}\text{Cs}$  into the plough layer as the surface horizons are removed by erosion and soil from depth is incorporated into the plough layer.

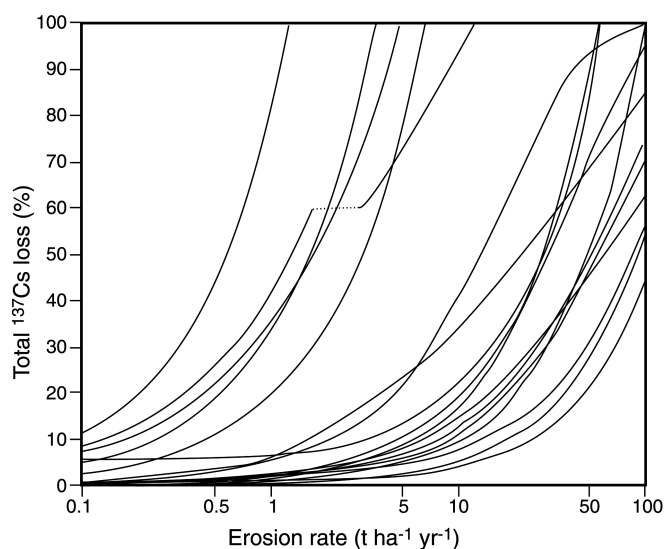


FIG. 4. Relationships between the percentage  $^{137}\text{Cs}$  inventory loss and soil erosion rate for a hypothetical soil derived from a range of existing calibration models and relationships documented in the literature (based on Walling and Quine (13)).

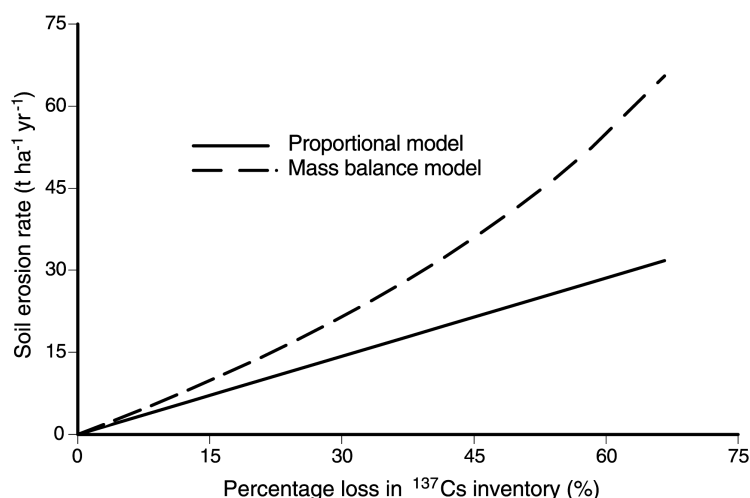


FIG. 5. A comparison of the relationship between soil erosion rate and percentage  $^{137}\text{Cs}$  inventory loss derived using the proportional model with that derived using a mass balance model.

Recent work has addressed the uncertainties associated with calibration procedures by developing improved theoretical models of the fate of  $^{137}\text{Cs}$  in eroding soils and by promoting a degree of standardization in the selection and application of calibration models. The improved models have attempted to incorporate a wide range of mechanisms that are likely to influence the precise relationship between erosion rate and reduction in the  $^{137}\text{Cs}$  inventory. These include grain size, selectivity of erosion and transport processes, preferential association of  $^{137}\text{Cs}$  with the finer fractions, the potential for removal of a proportion of the fallout prior to its incorporation into the plough layer by tillage, the role of tillage in causing the redistribution of soil and  $^{137}\text{Cs}$ , and the influence of slow downward diffusion and migration on the shape of the vertical profile of  $^{137}\text{Cs}$  in uncultivated soils [36–41].

A move towards standardization was promoted within the activities of the IAEA Co-ordinated Research Programmes on the use of environmental radionuclides in soil erosion and sedimentation investigations, whereby a number of calibration models were documented, and standardized software for the implementation of these models was made available to participants in the CRP and other interested workers [36]. In this situation, identical calibration procedures can be employed in different studies, thereby ensuring direct comparability of results. Nevertheless, there remains an important need to test the validity of these and similar theoretically based calibration relationships against independent measurements of erosion rates. Such independent data are difficult to assemble, but they could be provided by long term records of sediment output from erosion plots or small catchments, where there has been little change in management practice over the period involved.

#### *2.3.3. The role and significance of tillage translocation*

Early work on the use of  $^{137}\text{Cs}$  measurements to estimate soil erosion rates focused on the role of precipitation and runoff and associated erosion mechanisms in redistributing the fallout. Most of the calibration procedures discussed above implicitly assume that such mechanisms are primarily, if not solely, responsible for soil and associated  $^{137}\text{Cs}$  redistribution. More-recent work has, however, emphasized the potential role of tillage per se in redistributing soil and  $^{137}\text{Cs}$  [42,43]. Whenever soil is cultivated, tillage translocation, involving the lateral displacement of the cultivated horizon, will occur. Tillage erosion represents the net loss of soil due to tillage alone and this will occur at locations in the landscape characterized by changing rates of tillage translocation. Such locations include slope convexities where slope angles increase downslope. Similarly, tillage deposition (net accumulation of soil) will occur in slope concavities. Recognition of the potential role of tillage translocation in the redistribution of soil and  $^{137}\text{Cs}$  in cultivated areas has been reflected in investigations in two main ways. Firstly,  $^{137}\text{Cs}$  measurements have themselves been used to demonstrate the importance of tillage erosion and deposition in cultivated fields [44,45]. In this case, some features of the spatial pattern of  $^{137}\text{Cs}$  inventories within a field have been shown to be unrelated to the likely pattern of water erosion, but can be readily accounted for by tillage translocation. This is particularly the case for the reduced  $^{137}\text{Cs}$  inventories frequently found along ridge tops and at the upper boundaries of fields. Secondly, tillage translocation has now been incorporated into some of the calibration algorithms used to convert  $^{137}\text{Cs}$  measurements to estimates of erosion and deposition rates, so as to isolate the water-erosion component [46,47]. Such developments introduce additional complexity into the calibration calculations and could be seen as generating further uncertainty. Nevertheless, they attempt to take account of important processes, which will necessarily influence the relationship between  $^{137}\text{Cs}$  inventories and rates of soil redistribution associated with water erosion and should, therefore, be taken into account.

#### *2.3.4. Chernobyl $^{137}\text{Cs}$ and investigation of soil erosion*

The original application of  $^{137}\text{Cs}$  measurements in soil erosion investigations was based on study of the post-fallout redistribution of bomb-derived  $^{137}\text{Cs}$  within the landscape, and it provided the basis for nearly all subsequent work. The Chernobyl accident in 1986 was responsible for additional fallout of  $^{137}\text{Cs}$  over large areas of Eastern, Central, Northern and Western Europe. The existence of two fallout inputs, one spread over a decade or more and one occurring over a few days, more than 20 years later, introduced major complications into the simple basis for using  $^{137}\text{Cs}$  measurements to document soil

redistribution rates in areas receiving significant levels of Chernobyl fallout. In the years immediately following 1986, it was possible to separate the total  $^{137}\text{Cs}$  inventory found in a soil into bomb- and Chernobyl-derived components, by measuring the  $^{134}\text{Cs}$  inventory and calculating the accompanying  $^{137}\text{Cs}$  fallout input. Caesium-134 was not present in bomb fallout and measurements taken shortly after the Chernobyl accident indicated that  $^{137}\text{Cs}$  and  $^{134}\text{Cs}$  were present in that fallout in fixed proportions. This approach permitted quantification of the bomb fallout component and application of standard procedures for estimating soil-redistribution rates to the bomb-fallout data. However, the short half-life of  $^{134}\text{Cs}$  (2 years) has meant that it is no longer applicable, and the two components of  $^{137}\text{Cs}$  input cannot now be separated in areas that received Chernobyl fallout. Although there have been few attempts to do so, it is theoretically still possible to estimate soil redistribution rates by comparing the deviation of contemporary inventories from the current reference inventory, providing the magnitude of the bomb-fallout component of the total  $^{137}\text{Cs}$  inventory can be estimated from data available from the pre-Chernobyl period. In this case, records of both bomb- and Chernobyl-derived fallout inputs can be used as input to a mass balance calibration model. However, the additional complexity associated with taking account of two fallout inputs and the possibility that land-use practices and erosion rates were very different in the post-Chernobyl period mean that particular care is required in interpreting the results. The variability of Chernobyl fallout in response to local meteorological conditions at the time that the contaminated cloud passed over the area under investigation may introduce additional uncertainties.

In some areas that received substantial Chernobyl-derived  $^{137}\text{Cs}$  fallout, the total inventories are currently several orders of magnitude greater than the pre-existing bomb-derived inventories, in which case it is possible to interpret the current inventories solely in terms of the redistribution of the Chernobyl fallout in the period since 1986. For, example in the Lokna River basin in the Tula region of Central Russia, studied by Golosov and his co-workers [48,49], the  $^{137}\text{Cs}$  inventories measured in 1997 were of the order of  $500 \text{ kBq m}^{-2}$ , more than two orders of magnitude greater than the pre-existing bomb-derived inventories. These high levels permitted the use of a portable scintillation detector to measure  $^{137}\text{Cs}$  inventories directly in the field [50]. It proved possible to use measurements of Chernobyl-derived  $^{137}\text{Cs}$  inventories in a manner similar to that employing bomb-derived inventories elsewhere, and to investigate rates of erosion and deposition by studying both the vertical profiles of  $^{137}\text{Cs}$  in soil and sediment cores and the spatial variability of total inventories, which reflect the redistribution of the Chernobyl-derived fallout. In the Lokna River basin study, however, it was necessary to take account of spatial variability of the Chernobyl fallout and, thus, the reference inventory [51]. This was found to vary systematically across the catchment, increasing from ca. 300 to  $500 \text{ kBq m}^{-2}$  over a distance of about 1.75 km. In addition, the relatively short period since the input of Chernobyl fallout (i.e. ca. 11 years) meant that, in areas with low to moderate erosion rates, the inventories were only marginally reduced by erosion. In this situation, the validity of both the approach and the resulting estimates of erosion rates will increase as the time elapsed since 1986 increases. In areas of deposition, which tend to be localized within the catchment and include locations such as the balka bottoms,  $^{137}\text{Cs}$  inventories were significantly increased and showed clear evidence of sediment accumulation.

### *2.3.5. The IAEA Co-ordinated Research Projects*

In parallel with the methodological advances and refinements outlined above, the launching of two Co-ordinated Research Projects (CRPs) by IAEA in 1996 resulted in major contributions to co-ordinating and standardizing the procedures employed by researchers using  $^{137}\text{Cs}$  measurements in soil erosion and sedimentation investigations. The two closely linked CRPs, one (D1.50.05) led by the Soil and Water Management & Crop Nutrition Section of the Joint FAO/IAEA Division and concerned primarily with soil erosion, and the other (F3.10.01) led by the Isotope Hydrology Section of the Division of Chemical and Physical Sciences of IAEA and dealing mainly with sedimentation, were conceived at an Advisory Group Meeting held in Vienna in 1993. The aims and objectives of the two CRPs were formalized at a Consultants Meeting in Vienna in 1995 [52], and these emphasized the need to promote the standardization of field and laboratory procedures and interpretation methods for  $^{137}\text{Cs}$  studies. By bringing together representatives of some twenty-five research groups from various parts of the world to workshops held in Vienna in 1996, Bucharest in 1998, and Barcelona in 1999, the

CRPs successfully promoted the interchange of experience and ideas and the development of standardized procedures, for such aspects as selection of reference sites, the planning of sampling networks, laboratory analyses including inter-laboratory comparisons, and the use of calibration models. In the latter case, standard computer software for the application of a range of calibration models was developed and made available to all participants in the CRPs and to other interested parties [36]. In addition, IAEA has supported exchange of scientists among the various research groups, and the training of younger researchers at laboratories with experience in the application of the  $^{137}\text{Cs}$  technique.

## 2.4. Expanding applications

Much of the initial work on the use of  $^{137}\text{Cs}$  measurements in soil erosion investigations focused on validating the approach in various environments and areas of the world, and on developing and refining procedures. As the efficacy and value of the technique have been increasingly demonstrated, attention has turned to its application within a wide range of studies, as a means of assembling information both on the magnitude of soil redistribution rates and on the spatial patterns involved. These applications have, in turn, expanded, as the essentially unique opportunities offered by the  $^{137}\text{Cs}$  technique have been increasingly recognized. Two examples of these expanding applications can usefully be introduced. The first involves the use of the  $^{137}\text{Cs}$  technique in studies of erosion / crop-productivity relationships and the second relates to its use in validating distributed soil erosion and soil delivery models.

### 2.4.1. Exploring erosion / crop-productivity relationships

It is well known that, for most soils, crop production will decline as the upper parts of the soil profile are progressively lost by erosion. This decline in productivity reflects reduced rooting depth and degradation of the nutrient and moisture status of the soil. Precise information on the relationship between crop productivity and soil loss is, however, an important requirement for establishing soil-loss tolerance, for assessing the economic impact of erosion and the benefits of soil-conservation measures, and for forecasting the future impact of erosion on the production of food and fodder. Establishment of reliable relationships is, nevertheless, difficult due to the need for precise complementary information on both erosion rates and crop yields. Approaches to assembling such information have included the artificial removal of specified depths of topsoil from plots on which crops are grown and the use of traditional erosion plots to provide estimates of the erosion rate and of crop production [53]. In the former case, the representativeness of the experimental treatment can be seriously questioned. In the latter, the establishment of erosion plots commonly represents a substantial long term investment and the representativeness of an artificially bounded plot, on which it is possible to grow only a small area of the crop, can again be questioned. The accurate assessment of crop productivity can introduce further difficulties.

Although its potential has not yet been fully exploited, the  $^{137}\text{Cs}$  technique would appear to offer considerable scope for exploring soil erosion / crop productivity relationships, by providing a means of assembling retrospective information on medium-term rates of soil loss within a field, which could, in turn, be related to the current level of crop productivity. In such a situation, the soils are not artificially manipulated and it is possible to use representative areas of a particular crop within the “natural” landscape [e.g. 54]. The results obtained will be representative of the longer-term history of soil erosion and the balance between soil loss and soil formation. In addition, the opportunity to collect spatially distributed information on erosion rates, representative of an entire field, affords a valuable opportunity to make use of recent advances in precision recording of crop yields by mechanical harvesting equipment. In the case of cereal harvesters, for example, it is possible to couple a continuous record of yield, generated by monitoring the grain throughput, with information on the location of the harvester within the field recorded by a GPS, to provide a map of grain yield. Estimates of rates of soil loss obtained for specific points within the field can then be coupled with values of grain yield abstracted from the map or digital record, in order to provide the necessary information both on soil erosion rate and on crop yield.

The potential of this approach can be demonstrated from results of a pilot study undertaken by the author at Grickstone Farm, Chipping Sodbury, near Bristol, UK. Attention was directed to a single 12.9-ha cultivated field (Oldfield) (Fig. 6). Bulk soil cores were collected from two transects as shown, and sectioned cores were obtained from four additional points selected to be representative of the topography within the field. The sectioned cores confirmed the expected behaviour of  $^{137}\text{Cs}$  in an erosional situation, with core A evidencing an inventory close to the local reference inventory and a plough depth equivalent to a mass depth of ca.  $150 \text{ kg m}^{-2}$ . In contrast, core B, located at the bottom of a small valley, was characterized by an inventory nearly 40% greater than that for core A and by significant levels of  $^{137}\text{Cs}$  to a mass depth of ca.  $250 \text{ kg m}^{-2}$ . The values of  $^{137}\text{Cs}$  inventory obtained for the forty bulk cores collected along the two transects were converted to estimates of erosion and deposition rates using a mass balance calibration model; they provided evidence of erosion and deposition rates of up to  $15 \text{ t ha}^{-1} \text{ yr}^{-1}$ . The fields had been used for winter barley for several years and information on the variation of crop yield within the field for the 1996 harvest was obtained from the farmer (Fig. 7).

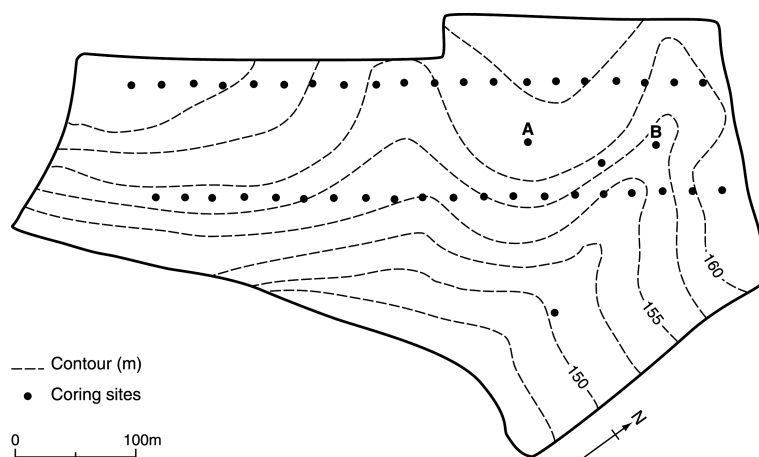


FIG. 6. The field at Grickstone Farm used to explore soil erosion / crop productivity relationships and the location of the points from which soil cores were collected, in order to estimate soil-redistribution rates.

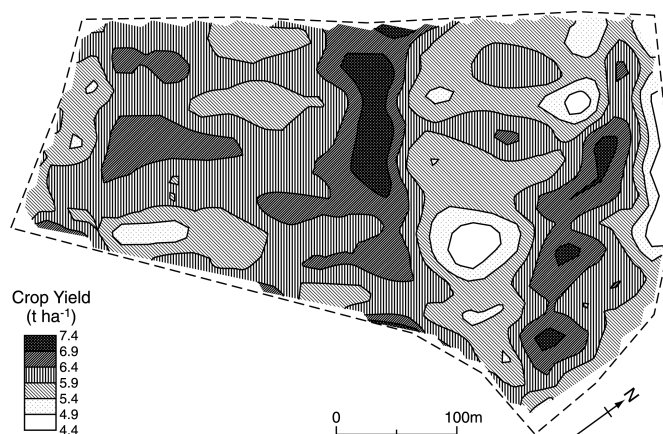


FIG. 7. Variation in yield of winter barley within the study field at Grickstone Farm, 1996 harvest.



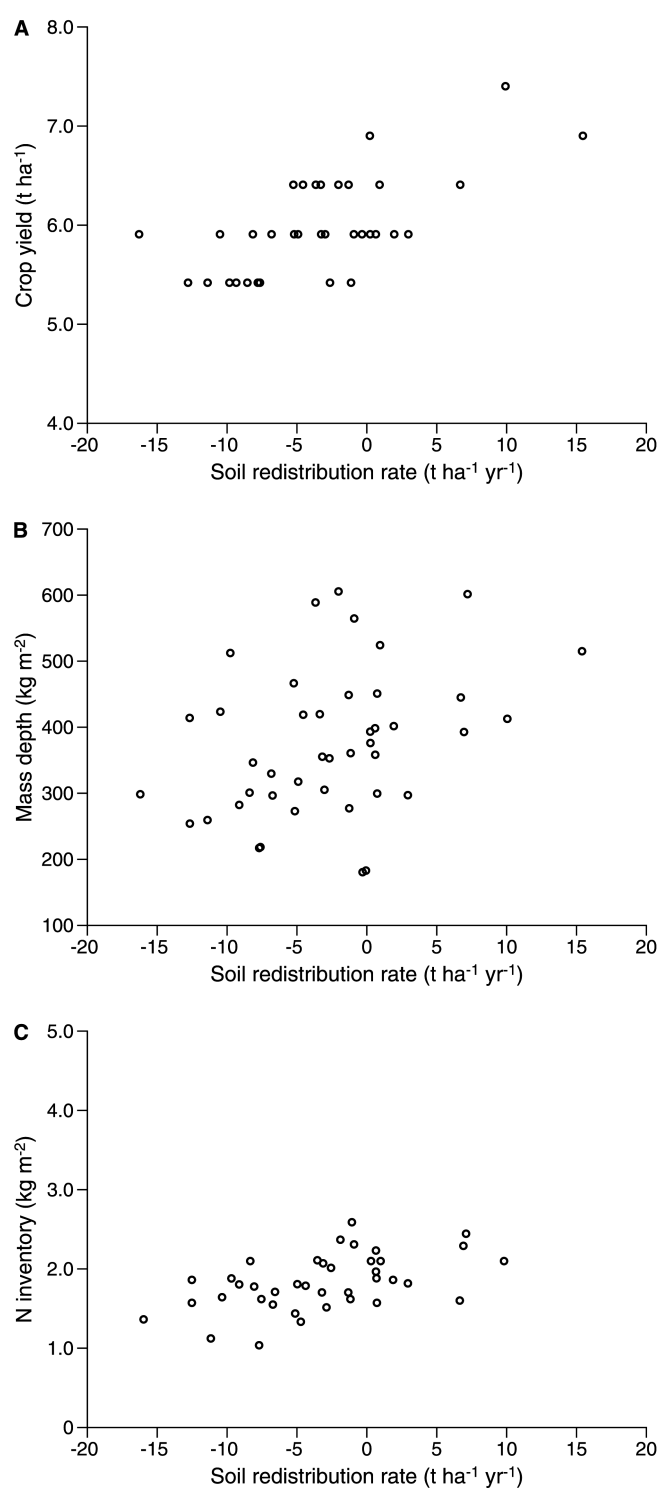


FIG. 8. The relationship between crop yield and soil-redistribution rate for the study field, and associated relationships between soil depth and soil N content and soil-redistribution rate.

The map in Fig. 7 shows clear evidence of differential yields, ranging from 4.4 to 7.4 t ha<sup>-1</sup>. A preliminary attempt to explore the relationship between crop productivity and soil-redistribution rates is shown in Fig. 8. Figure 8A shows a clear relationship, with minimum yields occurring in areas with the highest erosion rates and maximum yields in areas characterized by high rates of deposition. Since the erosion and deposition rates estimated from the <sup>137</sup>Cs measurements represent mean values for the past ca. 40 years, it is clear that the medium-term erosional history of the field now exerts a significant influence on crop yields.

The soils in the study field are relatively thin and overlay limestone bedrock. Measurements of soil depth were taken at each point where soil cores were collected, and Fig. 8B demonstrates a relationship between soil depth and soil-redistribution rate, with minimum depths occurring in areas with high erosion rates and maximum depths in areas with high deposition rates. Depth will influence both the moisture-retaining capacity and nutrient status of the soil, which will in turn influence crop yields. Figure 8C illustrates a clear relationship between soil nutrient status and soil-redistribution rate for the study fields, with eroded areas characterized by reduced nutrient content, and this undoubtedly exerts an important control on crop productivity.

#### *2.4.2. Validating and calibrating distributed soil erosion and soil delivery models*

Recent work on the development of distributed soil erosion and soil delivery models for small catchments and larger river basins has highlighted a situation where the ability to produce and refine such models has outstripped the capability to validate and test them. Traditional monitoring techniques, such as erosion plots, are unable to provide the distributed data on soil-redistribution rates within a landscape required for model testing and, in consequence, model validation has frequently been restricted to comparing measured and simulated sediment yields, rather than rates of sediment mobilization and redistribution within the modelled area. Correspondence of measured and modelled outputs is, in itself, an inadequate test of a model, since a sediment yield of a particular magnitude could be obtained from a wide range of combinations of erosion and deposition rates. For example, a low sediment yield could reflect either low erosion rates and efficient sediment delivery or high erosion rates and a low soil delivery ratio. In this situation, it is important to compare the erosion and deposition rates simulated by a model with independent information on those rates provided by field measurements, in order to confirm or refute the internal working of the model.

The  $^{137}\text{Cs}$  approach offers an essentially unique opportunity to assemble information for use in validating and developing distributed soil erosion and soil delivery models. This potential was recognized by De Roo, who has reported the successful use of estimates of erosion and deposition rates obtained from  $^{137}\text{Cs}$  measurements to test the ANSWERS model [55,56]. In such applications, the information on rates and patterns of soil redistribution generated from  $^{137}\text{Cs}$  measurements can be directly compared with the equivalent output from the model. Walling and He [57] have also reported an alternative approach to model validation and calibration involving a simple topographically driven model of soil redistribution. In this case, the model was adapted to simulate the redistribution of  $^{137}\text{Cs}$ , as well as of soil, and a comparison was made between the modelled pattern of  $^{137}\text{Cs}$  redistribution and that measured in the field. In this case, it was possible to calibrate several parameters of the redistribution model by optimizing the correspondence between measured and modelled values of  $^{137}\text{Cs}$  inventory. One important limitation of soil-redistribution data derived from  $^{137}\text{Cs}$  measurements for model validation and calibration is, however, its medium-term nature. In this regard it is not possible to consider individual events or even years. The validation must be based on a much longer period, equivalent to the timescale covered by  $^{137}\text{Cs}$  measurements. In the absence of the necessary longer-term input data for the model (e.g. precipitation data), it may be necessary to use a synthetic input series, or a representative short term record, and to emphasize correspondence of patterns rather than the absolute magnitude of soil-redistribution rates.

Figure 9 provides an example of the potential for using information derived from  $^{137}\text{Cs}$  measurements to test existing erosion and soil delivery models for a small catchment embracing a 7.5-ha area of a single field at Aller Barton Farm. The field concerned is that illustrated in Fig. 1 and the test involved the AGNPS [58,59] and ANSWERS [60] models. In order to apply these models to the field, it was discretized into  $11 \times 11$  m grid cells, providing a total of 620 cells. The AGNPS and ANSWERS models were then run for a hypothetical rainfall event, with a total precipitation of 18 mm within 3.5 h, which was selected as representative of a major erosion-generating event in the study area. The soil redistribution rates simulated by the models are presented in Fig. 9 and can be compared with the estimates of longer-term soil redistribution rates within the field provided by  $^{137}\text{Cs}$  measurements, also presented in Fig. 9.

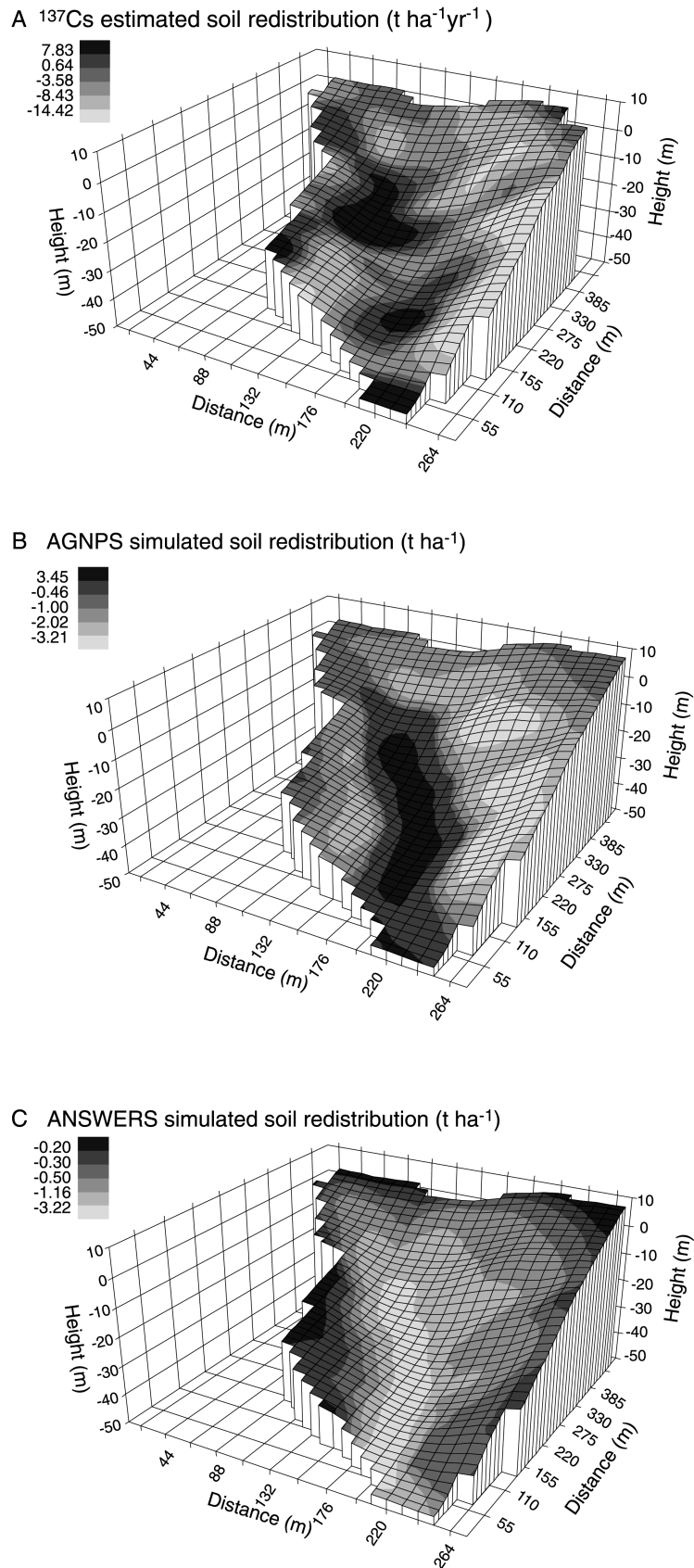


FIG. 9. Comparison of the pattern of longer-term soil redistribution within the field at Aller Barton Farm derived from  $^{137}\text{Cs}$  measurements (A) with the patterns simulated for a representative erosion event using the AGNPS (B) and ANSWERS (C) models.

In this situation, it is not possible to make a direct comparison between the absolute magnitude of the erosion rates simulated by the models and those estimated from the  $^{137}\text{Cs}$  measurements, but emphasis can be placed on the ability of the models to reproduce the main features of the spatial pattern demonstrated by the soil-redistribution estimates derived from the  $^{137}\text{Cs}$  measurements. In this context, significant difference can be seen to exist between the two models. In the case of the AGNPS model, there is a reasonable degree of similarity between the pattern of soil redistribution simulated by the model and that derived from the  $^{137}\text{Cs}$  measurements. More particularly, maximum erosion rates are seen to occur on the convex midslopes, lower erosion rates are found on the upper slopes, and there is evidence of deposition along the valley floor. In contrast, the ANSWERS model fails to reproduce the key features of the pattern associated with the  $^{137}\text{Cs}$  measurements. In this case the model shows erosion rates increasing downslope towards the outlet of the field and there is no evidence of deposition in the slope concavities. The soil delivery ratio predicted for the fields by the ANSWERS model is close to 100%, whereas that predicted by the AGNPS model is 48%. The latter is in close agreement with the value of 40% listed in Table I for the study field. This test of the AGNPS and ANSWERS models has limitations, in that the soil-redistribution rates derived from the  $^{137}\text{Cs}$  measurements represent estimates of medium-term mean annual rates (i.e. for a ca. 40-year period), whereas those obtained from the models are for a single hypothetical event. Nevertheless, comparison of the main features of the predicted pattern of soil redistribution within the field with that based on the  $^{137}\text{Cs}$  measurements demonstrates that, whereas the AGNPS model appears to provide meaningful results for the study area, the ANSWERS model does not. Further tests could make use of actual or synthetic long term rainfall series to obtain predictions of the magnitude and pattern of long term erosion rates within the field which would be more directly comparable with the data obtained from the  $^{137}\text{Cs}$  measurements. The unique potential of  $^{137}\text{Cs}$  measurements to provide distributed data for use in validating and testing distributed erosion and soil delivery models is, however, clearly demonstrated.

### 3. EXTENSION OF THE APPROACH TO INCLUDE OTHER FALLOUT RADIONUCLIDES

Although most work involving the use of environmental radionuclides in soil erosion investigations has focused on radiocaesium, attention has also been directed to the potential for using other radionuclides, both as an alternative and as a complement to  $^{137}\text{Cs}$ . One significant limitation of  $^{137}\text{Cs}$  is the relatively low inventories found in some areas of the world, more particularly equatorial regions and parts of the southern hemisphere. These low inventories, which can be an order of magnitude less than those found in the mid-latitudes of the northern hemisphere, can introduce problems in terms of detection limits and need for long count times. Furthermore, radioactive decay will reduce existing low inventories further in the future. Another potential limitation, noted above, is the additional complexity introduced by Chernobyl fallout, where the bomb-derived component of the inventory still accounts for a significant proportion of the total inventory. In such situations, interpretation of the  $^{137}\text{Cs}$  measurements needs to take account of both the different time periods associated with the two components of  $^{137}\text{Cs}$  fallout and differences in the nature of the fallout between the two periods. Whereas the Chernobyl fallout will have been deposited within a few days, bomb-derived fallout will have arrived over more than a decade. In these situations, excess  $^{210}\text{Pb}$  can provide an alternative tracer for documenting soil-redistribution rates. In addition, because the annual fallout of excess  $^{210}\text{Pb}$  can be considered to be essentially constant through time, in contrast to  $^{137}\text{Cs}$  where the fallout was effectively restricted to a specific period extending from the 1950s through to the 1970s, there is potential to use the two radionuclides in combination to obtain additional information on the erosional history of a site. Although, the ability of the  $^{137}\text{Cs}$  technique to provide estimates of medium-term average soil-redistribution rates is commonly seen as an advantage, due to their increased representativeness, this averaging could also be seen as a limitation. It is, for example, impossible to document changes in erosion rates associated with land-use change or to document the impact of a specific erosional event. It is this limitation that has been addressed by the use of  $^7\text{Be}$  as an alternative tracer. Due to its short half-life (53 days) it is possible to obtain information on soil-redistribution rates over very much shorter timescales. The application of both excess  $^{210}\text{Pb}$  and  $^7\text{Be}$  in investigations of erosion will be considered in more detail below.

### 3.1. The use of excess $^{210}\text{Pb}$ in soil erosion investigations

In contrast to  $^{137}\text{Cs}$ ,  $^{210}\text{Pb}$  is a naturally occurring radionuclide. It is a natural product of the  $^{238}\text{U}$  decay series, derived from the decay of gaseous  $^{222}\text{Rn}$  (half-life 3.8 d) the daughter of  $^{226}\text{Ra}$  (half-life 1,622 yr). Radium-226 exists naturally in soils and rocks. The  $^{210}\text{Pb}$  in soils generated in situ by the decay of  $^{226}\text{Ra}$  is termed *supported*  $^{210}\text{Pb}$  and is in equilibrium with  $^{226}\text{Ra}$ . However, upward diffusion of a small portion of the  $^{222}\text{Rn}$  produced in the soil and rock introduces  $^{210}\text{Pb}$  into the atmosphere and its subsequent fallout provides an input of this radionuclide to surface soils and sediments that will not be in equilibrium with its parent  $^{226}\text{Ra}$  and which is therefore termed *unsupported* or *excess*  $^{210}\text{Pb}$ , to distinguish it from that produced in situ by the decay of  $^{226}\text{Ra}$ . The amount of excess  $^{210}\text{Pb}$  in a soil or sediment sample can be calculated by measuring both the  $^{210}\text{Pb}$  and  $^{226}\text{Ra}$  activities, and subtracting the  $^{226}\text{Ra}$ -supported  $^{210}\text{Pb}$  component from the total  $^{210}\text{Pb}$  in the sample. The required measurements of  $^{210}\text{Pb}$  and  $^{226}\text{Ra}$  can be made by direct gamma spectrometry using low-energy, low-background HPGe detectors (cf. Ref. [61]). The annual deposition flux of excess  $^{210}\text{Pb}$  can be viewed as effectively constant through time. As with  $^{137}\text{Cs}$ , this fallout has been shown to be rapidly and firmly fixed by the surface soil [62], and excess  $^{210}\text{Pb}$  can thus be used as a tracer in a manner similar to that for  $^{137}\text{Cs}$ .

Successful attempts to use excess  $^{210}\text{Pb}$  in soil erosion investigations have been reported by Wallbrink et al. [63] and Walling et al. [64,65]. In the first case, the approach was based on measurements both of  $^{137}\text{Cs}$  and of excess  $^{210}\text{Pb}$ , and involved interpretation of the ratio of the excess  $^{210}\text{Pb}$  inventory to that for  $^{137}\text{Cs}$ . The work was undertaken within a forest area subjected to logging, which must be seen as special case due to the existence of uncultivated soils and a rapid shift from stable to eroding conditions as a result of forest clearance. The approach adopted by Walling and his co-workers is applicable more generally to cultivated soils. Following a similar procedure to that employed for  $^{137}\text{Cs}$ , they compared measured excess  $^{210}\text{Pb}$  inventories with the local reference inventory to identify areas of erosion and deposition, and they used a mass balance model to provide calibration relationships for converting the measured inventories to rates of erosion and deposition [65]. Due to the effectively continuous nature of the  $^{210}\text{Pb}$  fallout input, it is not possible to define an exact period within which the erosion has occurred and the mass balance model has been run with the assumption that erosion has been continuous for a period of 100 years. Where the onset of erosion can be dated more precisely, for example when the date of land clearance is known, the time period can be reduced or changed.

The results of applying this approach to the cultivated field at Aller Barton Farm shown in Fig. 1 are presented in Fig. 10. The mean erosion rate for the eroding areas within the field was estimated to be  $19.5 \text{ t ha}^{-1} \text{ yr}^{-1}$  and the mean deposition rate for depositional areas was  $22.1 \text{ t ha}^{-1} \text{ yr}^{-1}$ . The net erosion rate for the entire field was estimated to be  $4.4 \text{ t ha}^{-1} \text{ yr}^{-1}$ , giving a sediment delivery ratio of 41%. The example presented above relates to a cultivated field and further work is required to develop procedures for applying excess  $^{210}\text{Pb}$  measurements to estimate rates of soil redistribution in grassland and rangeland areas.

### 3.2. The use of $^7\text{Be}$ in soil erosion investigations

Like excess  $^{210}\text{Pb}$ ,  $^7\text{Be}$  differs from  $^{137}\text{Cs}$  in being of natural origin. It is produced primarily by the bombardment of the Earth's atmosphere by cosmic rays, and the annual fallout can again be viewed as essentially constant through time. Beryllium-7 is also rapidly and firmly fixed by the surface soil and offers potential as a tracer of soil redistribution. Its key characteristic is its short half-life of 53 days, which means that it is possible to study the erosion associated with individual events or short periods. Because of this, the influence of tillage can be separated from that of water erosion, since only the latter will be involved. Beryllium-7 is relatively easily measured using the same gamma detectors as for  $^{137}\text{Cs}$ . Reports of the successful use of  $^7\text{Be}$  in soil erosion investigations have been provided by Wallbrink and Murray in Australia [66] and Walling et al. in the UK [67,68], although the latter group was the first to document detailed procedures for estimating erosion and deposition rates. The approach to using  $^7\text{Be}$  to estimate erosion rates is essentially the same for both cultivated and undisturbed soils, since in the former case its application is limited to periods between cultivation episodes.

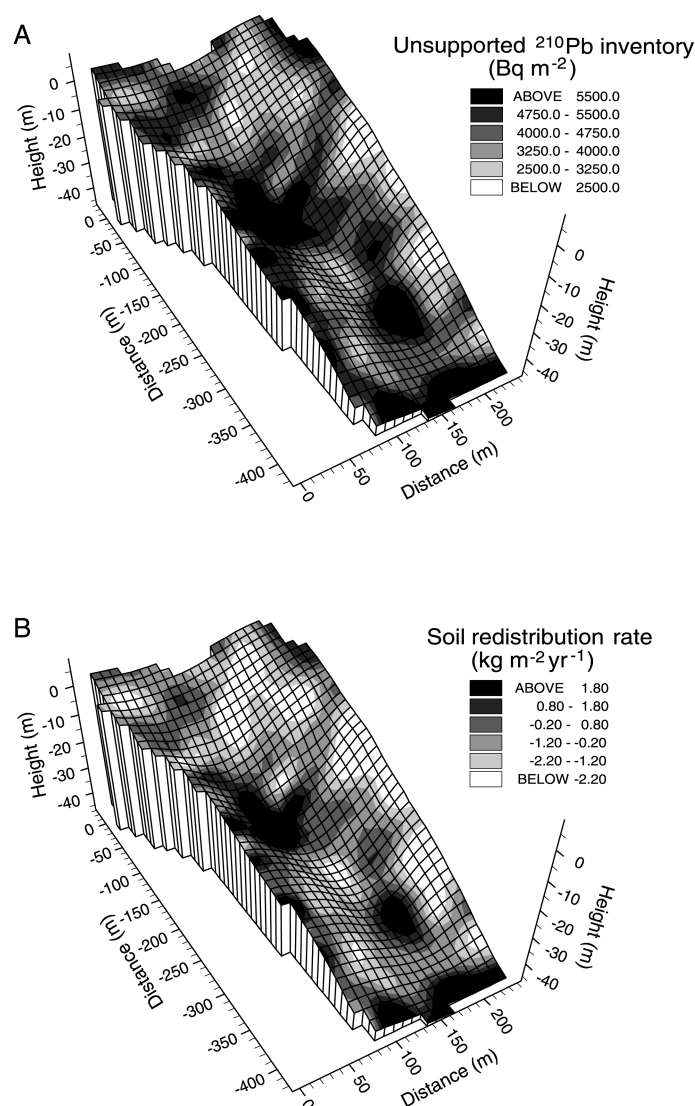


FIG. 10. The spatial distribution of excess  $^{210}\text{Pb}$  inventories (A) and soil-redistribution rates estimated from these inventories (B) within the study field at Aller Barton Farm.

The procedures developed by Walling et al. [67,68] are based on the fact that the  $^7\text{Be}$  fallout is found in a thin layer at, and immediately below, the soil surface, rarely extending below about 2 cm. The depth distribution of  $^7\text{Be}$  within this thin layer has been shown to be exponential, and this distribution can be characterized by its relaxation mass depth. As in the case of  $^{137}\text{Cs}$  and excess  $^{210}\text{Pb}$ , it is necessary to establish the local reference inventory and to establish the degree to which the inventories of individual soil cores have been depleted or enhanced relative to the reference value. In the case of eroded sites, it is possible to estimate the depth of soil removed from the degree of depletion of the inventory by establishing the exponential depth distribution that characterizes the local conditions. For depositional sites, the excess inventory is converted to an estimate of the deposition depth, by establishing a representative value for the  $^7\text{Be}$  content of sediment eroded from the upslope area and calculating the depth of sediment necessary to account for the excess inventory.

Although the short half-life of  $^7\text{Be}$  must be seen as its key advantage as a sediment tracer for use in soil erosion investigations, this also imposes certain restrictions on its effectiveness. More particularly, it is important to ensure that the reference inventory prior to an erosional event is spatially uniform, so that the spatial variability in the  $^7\text{Be}$  inventory measured after the event is a direct reflection of the pattern of erosion and deposition. This position will frequently be achieved under two conditions; firstly, in the period shortly after cultivation, when pre-existing  $^7\text{Be}$  will have been mixed into the

cultivation layer and surface concentrations will be near zero and, secondly, after an extended dry period when radioactive decay will have reduced  $^7\text{Be}$  concentrations in the surface soil to near zero. Equally, if cultivation or a dry period is followed by relatively low amounts of rainfall, which do not generate surface runoff and, therefore, soil redistribution, the reference inventory should remain spatially uniform until a larger rainfall event occurs.

Figures 11 and 12 present the results of a study carried out by the author and co-workers aimed at assessing the potential for using  $^7\text{Be}$  measurements to document short term erosion and deposition rates at Higher Walton Farm, shown in Fig. 1. The field had been sown to maize in the late spring of 1997, and the crop was harvested in early November 1997. The field remained uncultivated and the bare soil was susceptible to erosion. In early January 1998 a period of heavy rainfall (68.6 mm in 7 days) occurred and caused substantial erosion. This followed an extended period with limited rainfall and an absence of surface runoff, which produced the necessary condition of uniform  $^7\text{Be}$  inventories. Immediately after the period of heavy rainfall, the field was sampled for  $^7\text{Be}$ , by collecting shallow 15-cm-diameter cores from the intersections of a grid. A number of cores for depth-incremental sectioning were also collected both from the study field and from adjacent undisturbed sites to investigate the vertical distribution of  $^7\text{Be}$ .

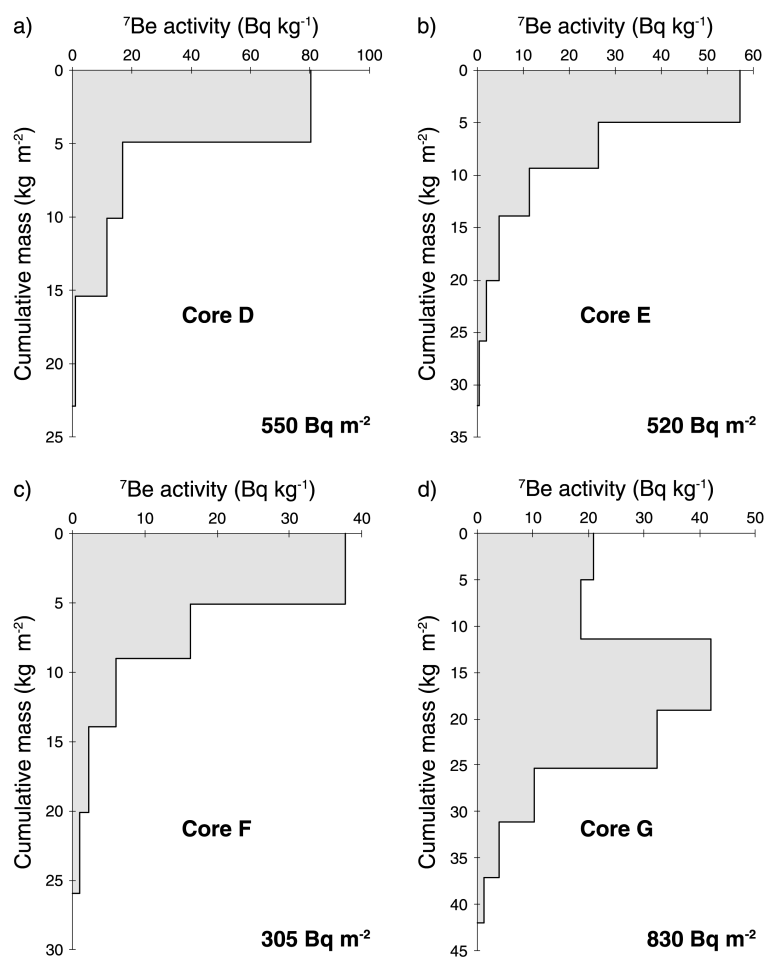


FIG. 11. The vertical distribution of  $^7\text{Be}$  activity within soil cores collected from an undisturbed site adjacent to the study field at Walton Farm (a) and from the study field itself (b, c, d).

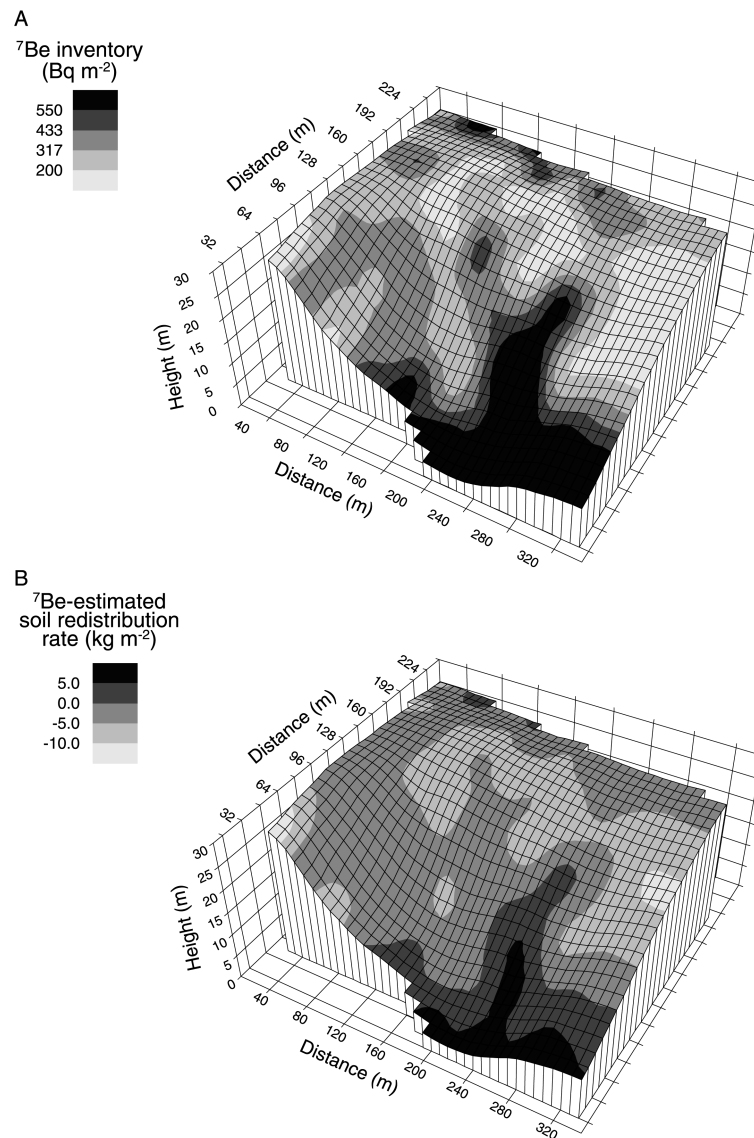


FIG. 12. The spatial distribution of  $^7\text{Be}$  inventories (A) and the estimates of soil-redistribution rates derived from these measurements (B) within the study field at Walton Farm.

Figure 11 depicts the depth distribution of  $^7\text{Be}$  in a core from an adjacent undisturbed stable site (core D), from an uneroded point on a flat area at the top of the field (core E), from an eroding site on the midslope (core F), and from a depression at the bottom of the field (core G). All four cores confirm the assumptions about the behaviour of  $^7\text{Be}$  outlined above. More particularly, cores D, E and F confirm the shallow exponential depth distribution and core G provides evidence of the burial of the  $^7\text{Be}$ -labelled surface by deposited sediment. The pattern of  $^7\text{Be}$  inventories found in the field after the erosion event, and the estimates of soil redistribution derived from these measurements, are shown in Fig. 12. These results must be seen as representing an important complement to those that have been obtained from the same field using  $^{137}\text{Cs}$  measurements, in that they enable the erosion rates associated with an individual event to be quantified and could thus afford a basis for testing event-based models.

#### 4. PERSPECTIVE

It is now about 30 years since  $^{137}\text{Cs}$  measurements were first used to estimate erosion rates. The technique has been developed and refined and, to an extent, standardized, and it has now been successfully employed in many parts of the world. Its application has recently been extended beyond the documentation of rates and patterns of soil loss to include the provision of erosion-rate data for use



in assessing erosion / productivity relationships and for the validation of spatially distributed soil erosion and soil delivery models. The approach has also been usefully expanded by making use of other environmental radionuclides, including excess  $^{210}\text{Pb}$  and  $^7\text{Be}$ , that can provide information relating to both shorter and longer timescales. It is clear that the  $^{137}\text{Cs}$  technique and its various developments now offer very considerable potential for use in soil erosion investigations. In many respects, the data that it can provide are unique and not obtainable using more traditional techniques. The activities of the IAEA and FAO in promoting the application and standardization of the technique in various parts of the world have played an important role in promoting its application. However, as the seriousness of soil erosion problems at global, regional and local scales is increasingly recognized, it deserves to be more widely applied. There is now a need to extend its use beyond that of a research tool and to integrate the technique into soil erosion assessment programmes on a more routine basis. Perhaps the greatest challenge for the future lies in upscaling the approach, which is essentially a means of obtaining point measurements of soil loss, in order to obtain information for large areas. Further work is required to couple the technique with remote sensing and to exploit the use of geostatistics in extrapolating point estimates across larger areas.

### ACKNOWLEDGEMENTS

The contributions of several colleagues and co-workers, including Qingping He, Tim Quine, Phil Owens, Will Blake and Jim Grapes to the results presented in this paper, and the support of the UK Natural Environment Research Council, Overseas Development Agency, Department for International Development, and the International Atomic Energy Agency for work on the application of fallout radionuclides in soil erosion investigations are gratefully acknowledged. Thanks are also extended to Mr. P. Lippiatt for providing the crop-yield data presented in Fig. 7 and for allowing access to the field at Grickstone Farm for collection of soil cores for  $^{137}\text{C}$  analysis.

### REFERENCES

- [1] BERNARD, J.M., IIVARI, T.A., Sediment damages and recent trends in the United States, *Int. J. Sediment Res.* **15** (2000) 135–148.
- [2] CLARK, E.H., et al., *Eroding Soils the Off-Farm Impacts*, The Conservation Foundation, New York (1985).
- [3] MOU, J., “Recent studies of the role of soil conservation in reducing erosion and sediment yield in the loess plateau area of the Yellow River Basin”, *Erosion and Sediment Yield: Global and Regional Perspectives* (WALLING, D.E., WEBB, B.W., Eds.), IAHS Publication no. **236**, IAHS Press, Wallingford (1996) 541–548.
- [4] LOUGHRAN, R.J., The measurement of soil erosion, *Prog. Phys. Geogr.* **13** (1990) 216–233.
- [5] EVANS, R., Some methods of directly assessing water erosion of cultivated land - a comparison of measurements made on plots and in fields, *Prog. Phys. Geogr.* **19** (1995) 115–129.
- [6] FERRO, V., MINACAPILLI, M. Sediment delivery processes at basin scale, *Hydrol. Sci. J.* **40** (1995) 703–717.
- [7] FOLLY, A., et al., Evaluation of the EUROSEM model using data from the Catsop watershed, The Netherlands, *Catena* **37** (1999) 507–519.
- [8] DE ROO, A.P.J., et al., Soil erosion modelling using ANSWERS and geographical information systems, *Earth Surf. Proc. Landforms* **14** (1989) 517–532.
- [9] RENARD, K.G., et al., RUSLE revisited: status, questions, answers and the future, *J. Soil Wat. Conserv.* **49** (1994) 213–220.
- [10] WALLING, D.E., QUINE, T.A., The use of caesium-137 measurements to investigate soil erosion on arable fields in the UK: potential applications and limitations, *J. Soil Sci.* **2** (1991) 147–165.
- [11] LOUGHRAN, R.J., et al., Estimation of soil erosion from caesium-137 measurements in a small cultivated catchment in Australia, *J. Appl. Radiat. Isot.* **39** (1988) 1153–1157.
- [12] MARTZ, L.W., DE JONG, E., Using caesium-137 to assess the variability of net soil erosion and its association with topography in a Canadian prairie province, *Catena* **14** (1987) 439–451.
- [13] WALLING, D.E., QUINE, T.A., Calibration of caesium-137 measurements to provide quantitative erosion rate data, *Land Degrad. Rehabilitat.* **2** (1990) 161–175.

- [14] WALLING, D.E., "Use of  $^{137}\text{Cs}$  and other fallout radionuclides in soil erosion investigations: progress, problems and prospects", Use of  $^{137}\text{Cs}$  in the Study of Soil Erosion and Sedimentation, IAEA-TECDOC-1028, IAEA, Vienna (1998) 39–62.
- [15] MENZEL, R.G., Transport of strontium-90 in runoff, *Science* **131** (1960) 499–500.
- [16] FRERE, M.H., et al., The loss of strontium-90 from small cultivated watersheds, *Soil Sci. Soc. Am. Proc.* **27** (1963) 82–83.
- [17] ROGOWSKI, A.S., TAMURA, T., Movement of  $^{137}\text{Cs}$  by runoff, erosion and infiltration on the alluvial Captina silt loam, *Health Phys.* **11** (1965) 1333–1340.
- [18] RITCHIE, J.C., et al., "The use of fallout cesium-137 as a tracer of sediment movement and deposition", *Mississippi Water Resour. Conf. Proc.* (1970) 149–163.
- [19] RITCHIE, J.C., et al., Estimating soil erosion from the redistribution of fallout Cs-137, *Soil Sci. Soc. Am. Proc.* **38** (1974) 137–139.
- [20] RITCHIE, J.C., McHENRY, J.R., Fallout Cs-137: A tool in conservation research, *J. Soil Wat. Conserv.* **30** (1975) 283–286.
- [21] LOUGHRAN, R.J. et al., "A reconnaissance survey of soil erosion in Australia", *Proc. 7th ISCO Conf. Sydney, Sept. 1992, Vol. 1*, (1992) 143–152.
- [22] WALLING, D.E., QUINE, T.A. "The use of caesium-137 measurements in soil erosion surveys", *Erosion and Sediment Transport Monitoring Programmes in River Basins (Proc. Symp. Oslo, 1992)* IAHS Publication no. **210**, IAHS Press, Wallingford (1992) 215–225.
- [23] BASHER, L.R. et al., Surface erosion assessment in the south Canterbury downlands, New Zealand using Cs-137 distribution, *Aust. J. Soil Res.* **33** (1995) 787–803.
- [24] GARCIA AGUDO, E., "Global distribution of  $^{137}\text{Cs}$  inputs for soil erosion and sedimentation surveys", Use of  $^{137}\text{Cs}$  in the Study of Soil Erosion and Sedimentation, IAEA-TECDOC-1028, IAEA, Vienna (1998) 117–121.
- [25] LARSEN, R.J., Worldwide Deposition of  $^{90}\text{Sr}$  Through 1983, Report EML-444, United States Department of Energy, New York (1985).
- [26] CAMBRAY, R.S., et al., Radioactive Fallout in Air and Rain: Results to the End of 1988, UK Atomic Energy Authority Report AERE-R 10155, HMSO, London (1988).
- [27] CAMPBELL, B.L. et al., "A method for determining sediment budgets using caesium-137", *Sediment Budgets (Proc. Symp. Porto Alegre, 1988)*, IAHS Publication no. **174**, IAHS Press, Wallingford (1988) 171–179.
- [28] WALLING, D.E., QUINE, T.A. Use of caesium-137 as a tracer of erosion and sedimentation: Handbook for the Application of the Caesium-137 Technique, Department of Geography, University of Exeter, UK (1993).
- [29] WALLBRINK, P.J. et al., "Measuring soil movement using  $^{137}\text{Cs}$ : implications of reference site variability", *Variability in Stream Erosion and Transport (Proc. Symp. Canberra, 1994)*, IAHS Publication No. 224, IAHS Press, Wallingford (1994) 95–102.
- [30] SUTHERLAND, R.A., Spatial variability of  $^{137}\text{Cs}$  and the influence of sampling on estimates of sediment redistribution, *Catena* **21** (1994) 57–71.
- [31] SUTHERLAND, R.A., Caesium-137 soil sampling and inventory variability in reference locations: a literature survey, *Hydrol. Proc.* **10** (1996) 43–53.
- [32] OWENS, P. N., WALLING, D.E., Spatial variability of caesium-137 inventories at reference sites: An example from two contrasting sites in England and Zimbabwe, *Appl. Radiat. Isot.* **47** (1996) 699–707.
- [33] WALLING, D.E., BRADLEY, S.B. "The use of caesium-137 measurements to investigate sediment delivery from cultivated areas in Devon, UK", *Sediment Budgets (Proc. Symp. Porto Alegre, 1988)*, IAHS Publication No **174**, IAHS Press, Wallingford (1988) 325–335.
- [34] BASHER, L.R., MATTHEWS, K.M., Relationship between  $^{137}\text{Cs}$  in some undisturbed New Zealand soils and rainfall, *Aust. J. Soil. Res.* **31** (1993) 655–663.
- [35] RUSE, M.E., PEART, M.R.,  $^{137}\text{Cs}$  reference site characteristics in Hong Kong — some considerations, *Phys. Chem. Earth (A)* **24** (1999) 887–891.
- [36] WALLING, D.E., HE, Q. Models For Converting  $^{137}\text{Cs}$  Measurements to Estimates of Soil Redistribution Rates on Cultivated and Uncultivated Soils. A Contribution to the IAEA Coordinated Research Programmes on Soil Erosion and Sedimentation, Department of Geography, University of Exeter, UK (1997).

- [37] WALLING, D.E., HE, Q., Improved models for estimating soil erosion rates from  $^{137}\text{Cs}$  measurements, *J. Environ. Qual.* **28** (1999) 611–622.
- [38] YANG, H., et al., Quantitative model of soil erosion rates using  $^{137}\text{Cs}$  for uncultivated soil, *Soil Sci.* **163** (1988) 246–257.
- [39] FREDERICKS, D.J., PERRENS, S.J., “Estimating erosion rates using caesium-137: II Estimating rates of soil loss”, *Sediment Budgets (Proc. Symp. Porto Alegre, 1988)*, IAHS Publication No. 174, IAHS Press, Wallingford (1988) 233–240.
- [40] OWENS, P.N., WALLING, D.E., The use of a numerical mass-balance model to estimate rates of soil redistribution on uncultivated land from  $^{137}\text{Cs}$  measurements, *J. Environ. Radioactivity* **40** (1998) 185–203.
- [41] ZHANG, X., et al., Simplified mass balance models for assessing soil erosion rates on cultivated land using caesium-137 measurements, *Hydrol. Sci. J.* **44** (1999) 33–45.
- [42] LINDSTROM, M.J., et al., Soil movement by tillage as affected by slope, *Soil Tillage Res.* **17** (1990) 255–264.
- [43] GOVERS, G., et al., The role of tillage in soil redistribution on hillslopes, *Eur. J. Soil. Sci.* **45** (1994) 469–478.
- [44] LOBB, D.A., et al., Tillage translocation and tillage erosion on shoulder slope landscape positions measured using  $^{137}\text{Cs}$  as a tracer, *Can J. Soil Sci.*, **75** (1995) 211–218.
- [45] QUINE, T.A. et al., Erosion processes and landform evolution on agricultural land new perspectives from caesium-137 data and topographic-based erosion modelling, *Earth Surf. Proc. Landforms* **22** (1997) 799–816.
- [46] QUINE, T.A., “Estimation of erosion rates from caesium-137 data: the calibration question”, *Sediment and Water Quality in River Catchments (FOSTER, I.D.L., et al., Eds.)*, Wiley, Chichester, UK (1995) 307–329.
- [47] QUINE, T.A. Use of caesium-137 data for validation of spatially distributed erosion models: the implications of tillage erosion, *Catena* **37** (1999) 415–430.
- [48] GOLOSOV, V.N., et al., Chernobyl  $^{137}\text{Cs}$  redistribution in the small basin of the Lokna River, Central Russia, *Phys. Chem. Earth (A)* **24** (1999) 881–885.
- [49] GOLOSOV, V.N., et al., “Post-fallout redistribution of Chernobyl-derived caesium-137 in small catchments within the Lokna River basin, Russia”, *The Role of Erosion and Sediment Transport in Nutrient and Contaminant Transfer (Proc. Symp. Waterloo, 2000)*, IAHS Publication No. 263, IAHS Press, Wallingford (2000) 49–57.
- [50] GOLOSOV, V.N., et al., Application of a field-portable scintillation detector for studying the distribution of  $^{137}\text{Cs}$  inventories in a small basin in central Russia, *J. Environ. Radioactivity* **48** (2000) 79–94.
- [51] GOLOSOV, V.N., et al., The spatial variability of Chernobyl-derived  $^{137}\text{Cs}$  inventories in a small agricultural drainage basin in central Russia, *Appl. Radiat. Isot.* **51** (1999) 341–352.
- [52] INTERNATIONAL ATOMIC ENERGY AGENCY, Use Of  $^{137}\text{Cs}$  in the Study of Soil Erosion and Sedimentation, IAEA-TECDOC-1028, Vienna (1998).
- [53] TENGBERG, A., et al., The impact of erosion on soil productivity — An experimental design applied in Sao Paulo State, Brazil, *Geografiska Annal.* **79A** (1997) 95–107.
- [54] MOULIN, A.P., et al., Spatial variability of wheat yield, soil properties and erosion in hummocky terrain, *Can. J. Soil Sci.* **74** (1994) 219–228.
- [55] DE ROO, A.P.J., Modelling Surface Runoff and Soil Erosion in Catchments Using Geographical Information Systems, University of Utrecht, Utrecht, the Netherlands (1993).
- [56] DE ROO, A.P.J., WALLING, D.E., “Validating the ‘ANSWERS’ soil erosion model using  $^{137}\text{Cs}$ ”, *Conserving Soil Resources, European Perspectives (RICKSON, R.J., Ed.)*, CAB International, Cambridge (1994) 246–263.
- [57] WALLING, D.E., HE, Q., “Use of fallout  $^{137}\text{Cs}$  measurements for validating and calibrating soil erosion and sediment delivery models”, *Modelling Soil Erosion, Sediment Transport and Closely Related Hydrological Processes, (Proc. Symp. Vienna, 1998)* IAHS Publication No. 249 (1998) 267–278.
- [58] YOUNG, R.R. et al., AGNPS, Agricultural non-point-source pollution model for evaluating agricultural watersheds, *J. Soil Wat. Conserv.* **44** (1989) 168–173.
- [59] PERRONE, J., MADRAMOOTOO, C.A., Sediment yield prediction using AGNPS, *J. Soil Wat. Conserv.* **54** (1999) 415–419.

- [60] BEASLEY, D.B., et al., Modelling sediment yield from agricultural watersheds, *J. Soil Wat. Conserv.* **37** (1982) 113–117.
- [61] JOSHI, S.R., Nondestructive determination of lead-210 and radium-226 in sediments by direct photon analysis, *J. Radioanal. Nucl. Chem. Art.* **116** (1987) 169–182.
- [62] HE, Q., WALLING, D.E. Interpreting particle size effects in the adsorption of  $^{137}\text{Cs}$  and unsupported  $^{210}\text{Pb}$  by mineral soils and sediments, *J. Environ. Radioact.* **30** (1996) 117–137.
- [63] WALLBRINK, P.J., MURRAY, A.S., Determining soil loss using the inventory ratio of excess lead-210 to caesium-137, *Soil Sci. Soc. Am. J.* **60** (1996) 1201–1208.
- [64] WALLING, D.E., et al., “Use of caesium-137 and lead-210 as tracers in soil erosion investigations”, *Tracer Technologies for Hydrological Systems*, (Proc. Symp. Boulder, 1995), IAHS Publication No. 229 (1995) 163–172.
- [65] WALLING, D.E., HE, Q. Using fallout lead-210 measurements to estimate soil erosion on cultivated land, *Soil Sci. Soc. Am. J.*, **63** (1999) 1404–1412.
- [66] WALLBRINK, P.J., MURRAY, A.S., Distribution and variability of  $^7\text{Be}$  in soils under different surface cover conditions and its potential for describing soil redistribution processes, *Water Resour. Res.* **32** (1996) 467–476.
- [67] BLAKE, W.H., et al., Fallout beryllium-7 as a tracer in soil erosion investigations, *Appl. Radiat. Isot.* **51** (1999) 599–605.
- [68] WALLING, D.E., et al., Use of  $^7\text{Be}$  and  $^{137}\text{Cs}$  measurements to document short- and medium-term rates of water-induced soil erosion on agricultural land, *Wat. Resour. Res.* **35** (2000) 3865–3874.

## COMPARISON OF $^{137}\text{Cs}$ FALLOUT REDISTRIBUTION ANALYSIS AND CONVENTIONAL EROSION-PREDICTION MODELS (WEPP, USLE)\*

G. SPAROVEK, O.O.S. BACCHI, S.B.L. RANIERI  
University of São Paulo,  
Piracicaba, Brazil

E. SCHNUG  
Institute of Plant Nutrition and Soil Science (FAL),  
Braunschweig, Germany

I.C. De-MARIA  
Agronomic Institute (IAC),  
Campinas, Brazil

### Abstract

Soil erosion is the most important component of the degradation of tropical agroecosystems. The rates of erosion should be considered in land evaluation and conservation planning assessment. The methods available for erosion prediction are not sufficiently well calibrated or validated for tropical soils, climates and crops. Thus, differences in estimated soil-erosion values may be expected, even if considering a single set of input data. Three methods for the estimation of soil erosion (USLE, WEPP, and  $^{137}\text{Cs}$ ) were applied to a watershed cultivated with sugarcane in southeastern Brazil. The absolute erosion-rate values and differences in the spatial distribution were evaluated. The overall results suggest important differences in the estimates obtained by the three methods. The differences occurred both in mean values and in geographic locations. The relative mean values for soil loss were  $\text{USLE} > ^{137}\text{Cs} > \text{WEPP}$  and for standard deviations were  $\text{USLE} > \text{WEPP} > ^{137}\text{Cs}$ , indicating that USLE predicted the highest erosion values spread out over the widest range. The poor geographical coincidence of the results is evidence that values resulting from non-calibrated erosion methods should be considered only as qualitative indications. The method selection should consider overall site variability in relation to factors to which the methods are known to be sensitive.

### 1. INTRODUCTION

In tropical agroecosystems, erosion is usually considered to be the most important aspect of soil degradation, especially where land use is intense [1]. Crop productivity can be affected either due to organic matter loss or to nutrient depletion [2]. Therefore, erosion rates should be considered as a component of land evaluation under tropical conditions. Soil conservation planning, land-use-technology-impact assessment, and the evaluation of the sustainability of agriculture are examples of issues that require estimates of erosion [3, 4].

Soil-erosion evaluations on the large scale, for example in a watershed, cannot be based on direct measurements due to methodological restrictions and temporal variability [5,6]. Therefore, under such conditions, erosion is usually estimated. If, instead of qualitative erosion-risk determination, quantitative predictions are desired, these estimations have to be based on models or on direct assessment, for example through  $^{137}\text{Cs}$ -fallout redistribution analysis.

The Universal Soil Loss Equation (USLE) [7] is the most comprehensive statistical method for the determination of soil erosion. However, it cannot be used to estimate sedimentation. The Water Erosion Prediction Project (WEPP) [8] is conceptually different because it is based on soil-erosion prediction, and estimates sedimentation as well. The  $^{137}\text{Cs}$ -fallout redistribution analysis estimates

---

\*This work was part of an FAO/IAEA Co-ordinated Research Project funded by the International Atomic Energy Agency (IAEA) BRA-8898 and was partially sponsored by FAPESP.

erosion directly, based on soil  $^{137}\text{Cs}$  activity. It is sensitive not only to soil-material redistribution via erosion, but also to other processes such as tillage, grading, and road construction [9, 10]. All three methods have been validated under specific experimental conditions, usually compared to measured erosion data resulting from natural or simulated rainfall [7, 9, 11]. Each of the methods is based on a different set of theoretical assumptions, and, in part, estimates different parameters (USLE estimates soil erosion rates, and WEPP and  $^{137}\text{Cs}$  estimate erosion and deposition rates). The equation parameters for USLE and WEPP, as well as the statistical procedures to convert  $^{137}\text{Cs}$  activity to erosion rates, were determined in temperate environments, mostly in North America and Europe, under soil, climate, management, and fallout conditions different from those found in tropical regions.

Probably, bias imposed by the individual methods results from differences both in theoretical assumptions and in the exogenous fundamental database when applied to tropical conditions. These biases may result in differences in estimates even when utilizing the same input data. The analysis of these differences, in magnitude and geographic location, is a step towards understanding the limitations and potentials of these approaches to determining erosion.

The objective of this study was to compare soil-erosion-prediction patterns using  $^{137}\text{Cs}$  fallout redistribution analysis with USLE and WEPP, in a watershed intensively cultivated with sugarcane in Brazil, i.e. conditions under which neither model was developed or validated.

## 2. MATERIAL AND METHODS

The study area is a 6-ha parcel of the Ceveiro watershed (2,200 ha in total) located in southeastern Brazil (Piracicaba, at 22°38'54"S, 47°45'40"W. Climate, according to Koeppen's classification is Cwa. The S-shaped slope profiles have a mean slope value of 16%. The soil, an Arenic Paleudult, has a surface layer composed of 70% sand decreasing to 50% in the subsurface Bt horizon. Local and overall land use is primarily intensive sugarcane production (4.8 ha or 80% at the site, and 50% regionally). In the central part of the area, an abandoned pasture (1.2 ha) acts as a buffer strip. Past soil erosion is evident by a mosaic of colours on the surface, extending from grey at the top slope positions characterizing the original surface horizon to yellow at the Bt horizon, originally at a depth of 1.0 m, at mid-slope. At the end of the slopes and under pasture, a deep surface unstructured sandy horizon, indicating recent depositions, dominates.

The  $^{137}\text{Cs}$  methodology was applied according to Ref. [10]. Samples were collected to a depth of 0.8 m, in order to include all  $^{137}\text{Cs}$  present in the soil profiles, on six transects with a distance of approximately 30 m between sampling positions. The  $^{137}\text{Cs}$  determinations were performed on gamma spectrometry equipment (detector model GEM-20180P, Pop Top EG & G ORTEC, associated with a multi-channel analyser; 1-L Marinelli beakers with counting time of 20 to 24 h). The conversion of  $^{137}\text{Cs}$  inventories ( $\text{Bq m}^{-2}$ ) into soil redistribution ( $\text{Mg ha}^{-1} \text{ yr}^{-1}$ ) was made by a proportional model as described in Ref. [12].

Slope information for USLE and WEPP was extracted from a topographic contour map, scale 1:10,000. Twenty-five soil-erosion-estimation transects were defined for USLE and WEPP calculations. The USLE was applied progressively to the intersections of the contour lines with each one of the twenty-five erosion-estimation transects. For WEPP, the altitude Z values were converted into relative slope values using an interface program for building the slope-input files. Local climate data collected daily for 30 years were used to calculate USLE and WEPP climate inputs. Soil erodibility for USLE (K factor) was based on equations suggested in Ref. [13] and computed from analytical results determined from soil samples collected at the same positions as for  $^{137}\text{Cs}$ -activity measurements, resulting in a value of  $0.0285 \text{ Mg h MJ}^{-1} \text{ mm}^{-1}$ . The same procedure was used for WEPP, computing the soil-input file based on the internal equations of WEPP version 99.5. Management files for sugarcane and pasture for WEPP were computed using the 99.5 version shell. The cover and management factor (C) and support practice factor (P) values for USLE were based on Ref. [14]. The combined C×P values were  $\text{CP}=0.1533$  for sugarcane and  $\text{CP}=0.0080$  for pasture.

The GIS procedures were carried out by means of TNTmips (Micro Images®) version 6.2. After soil-erosion determination, the values representing the sampling points for  $^{137}\text{Cs}$  analysis and the intersections of the altitude contour lines with the twenty-five transects were georeferenced. The interpolation procedure to transform the data to raster format (1.0×1.0 m pixel or 354 lines×348 columns) was the same for all three methods, i.e. squared inverse distance linear interpolation. The differences or residues for all model combinations ( $^{137}\text{Cs}$  minus USLE,  $^{137}\text{Cs}$  minus WEPP, and USLE minus WEPP) were calculated by subtraction of individual pixel values. General statistic mean, standard deviation, minimum and maximum values from soil loss, and subtracted amounts were calculated based on the complete set of interpolated values. None of the adopted methods were calibrated or validated under the tillage, soil and climatic conditions of the investigated watershed.

### 3. RESULTS AND DISCUSSION

Table I shows general results for each soil-erosion prediction method, and the differences (subtractions) between them. Table II shows the soil erosion and method subtraction erosion rate class percentages.

TABLE I. GENERAL STATISTICS AND EROSION/DEPOSITION FREQUENCIES

Method	Minimum	Maximum	Mean	Std. Dev.	Erosion (+)	Depos'n (-)
	(Mg ha <sup>-1</sup> yr <sup>-1</sup> )				(%)	
Predicted soil erosion						
<sup>137</sup> Cs	-86	59	28	16	93	7
USLE	0	435	52	39	100	0.0
WEPP	-831	146	13	20	84	16
Difference between methods						
<sup>137</sup> Cs-USLE	-405	53	24	41	31	69
<sup>137</sup> Cs-WEPP	-120	860	16	26	77	23
USLE-WEPP	-6	831	39	36	99.6	0.4

TABLE II. ESTIMATED EROSION CLASS AND METHOD-DIFFERENCE CLASS FREQUENCIES

Method	Class in Mg ha <sup>-1</sup> yr <sup>-1</sup>								
	>90	60 to 90	30 to 60	15 to 30	0 to 15	0 to -15	-15 to -30	-30 to -60	<-60
	(%)								
$^{137}\text{Cs}$	0	0	57	26	10	5	1	1	0
USLE	13	24	28	19	16	0	0	0	0
WEPP	0	0	6	48	29	10	4	2	1
	Class in Mg ha <sup>-1</sup> yr <sup>-1</sup>								
	>36	36 to 12	12 to 6	6 to -6	-6 to -12	-12 to -36	<-36		
	(%)								
$^{137}\text{Cs}$ -USLE	3	18	5	9	5	26	34		
$^{137}\text{Cs}$ -WEPP	20	33	15	16	6	8	2		
USLE-WEPP	44	32	10	14	0	0	0		

The overall results suggest important differences in soil-loss estimations among the three methods. The differences occurred both in mean values and in geographic performance. Erroneous basic assumptions may be contributing to the differing soil-erosion estimate patterns. A detailed discussion on specific aspects of the method performance and differences follows.

Relatively, the mean soil loss values were  $USLE > ^{137}Cs > WEPP$ , and the standard deviation values were  $USLE > WEPP > ^{137}Cs$ , indicating that USLE predicted the highest erosion values spread out over the widest range. The USLE has a very high sensitivity in relation to the topographic factor, which is composed of two sub factors related to slope length (L factor) and steepness (S factor) [15]. The USLE statistic database, used to develop the equation parameters, was based on uniform small plots (~22 m long, ~3 m wide) under experimental conditions. In these plots, no or little deposition is expected as compared to, longer, complex slopes in farmers' fields. Lack of consideration of sedimentation and of history of the experimental conditions of USLE development were suggested in Ref. [16] to explain over-prediction in relation to  $^{137}Cs$ . The conditions under which this study was conducted are similar to those described in Ref. [16] for USLE soil loss over-predictions, i.e. long and complex slopes under various management practices. The average slope length from the twenty-five soil-erosion-estimation transects was 129 m (six times longer than the ULSE standard experimental plots) with a minimum value of 78 m and maximum of 219 m. Higher soil-loss estimations for Revised Universal Soil Loss Equation (RUSLE) [17] as compared to WEPP were attributed to a lower sensitivity to crop-related parameters and higher sensitivity to topographic factors in the former [18]. A contrasting land-use and management pattern with an abrupt boundary between intensively cultivated sugarcane and an abandoned pasture over steep, long and complex S-shaped slopes are favourable conditions for over-estimations, or at least, higher erosion-rate estimations for the USLE as compared to WEPP or  $^{137}Cs$ . This results from the experimental conditions under which USLE was developed, its sensitivity in relation to topographic and management factors, and the lack of sedimentation prediction.

The possible influence of the erosion-rate estimation method in relation to land-use-planning decisions was significant. Considering  $12 \text{ Mg ha}^{-1} \text{ yr}^{-1}$  as a normally accepted soil-loss-tolerance value [19] the mean values would define the area as acceptable in relation to erosion rates with WEPP, but would be approximately two times the tolerable erosion rate with  $^{137}Cs$ , and some four times the tolerable rate with USLE. Common sense dictates that an area with a coloured soil-surface mosaic showing layers that were once 1-m deep in the original soil profile, sediments spread out on the lower slopes and frequent ephemeral gullies, is far from acceptable in relation to erosion. It is not advisable to rely on quantitative results when working with erosion-prediction methods under non-calibrated situations, if it is not possible to guarantee accuracy based on experimental results. External standards for acceptable or tolerable soil-erosion rates, in this case, resulted in unrealistic estimates. Under such conditions the tolerable value should be internally determined and based on common perceptions of problems or other indicators such as productivity decline, silting, or water pollution. Following this proposal, the relative geographical distribution pattern becomes more important than the absolute values yielded from the erosion-estimation methods.

WEPP estimated a continuous deposition area located at the final one-third of the transects, coincident with the transition of sugarcane to pasture and with lower slope values. The largest depositions, in this case, were found close after the crop transition (range of  $< -60 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ ), and deposition rates were in the range of 0 to  $-15 \text{ Mg ha}^{-1} \text{ yr}^{-1}$  at the last quarter of the transects. The high sensitivity of WEPP to crop parameters and the large differences with sugarcane in relation to pasture, surface roughness and tillage [18] are the reasons for WEPP reaction to a crop shift. USLE performance at the same boundary had a less significant soil-erosion rate reduction, because of higher sensitivity to slope factors and non-consideration of sedimentation [7, 15]. The geographic distribution of soil-erosion values estimated by  $^{137}Cs$  did not follow the classical, expected trend shown by WEPP and USLE. Common sense dictates increasing erosion rates from up to downslope and deposition (or erosion reduction) at the end slope where a pasture buffer strip rises on a smoother landscape position. The  $^{137}Cs$  redistribution analysis method is, however, sensitive to other soil-transport mechanisms, e.g. road construction or maintenance, surface levelling after gully formation and downhill ploughing [9]. All these operations presently occur in the area and can be considered as routine procedures under



intensive sugarcane cultivation, and thus undertaken in the area for the past 25 years. The indication of depositions side by side with high erosion rates at the upper slope for  $^{137}\text{Cs}$ , cannot be explained by basic concepts of soil erosion or process theory. However, these patterns can be understood by the fact that a main road was built at this location for sugarcane transportation and that, to level the road, soil material was scraped from one place (high soil erosion values) to another (deposition). Probably, this human-made redistribution did not export soil out of the area, therefore, in theory, it should not affect average erosion rate values. The intermediate performance of  $^{137}\text{Cs}$  in predicting erosion rates as compared to USLE (expected over-prediction in topographic conditions as observed in this study area) and WEPP (high sensitivity to non-calibrated crop parameters) may be meaningful.

The relative values for method differences were  $\text{USLE-WEPP} > ^{137}\text{Cs-USLE} > ^{137}\text{Cs-WEPP}$ . Positive values were observed with USLE-WEPP and  $^{137}\text{Cs-WEPP}$  and negative values with Cs-USLE (Table I). USLE estimated higher soil erosion values in 69% of the areas as compared to  $^{137}\text{Cs}$  and 99.6% in relation to WEPP. Estimates using  $^{137}\text{Cs}$  were higher than WEPP in 23% of the area. The difference class from 6 to  $-6 \text{ Mg ha}^{-1} \text{ yr}^{-1}$ , which would yield the same interpretation in relation to soil loss tolerance considering  $12 \text{ Mg ha}^{-1} \text{ yr}^{-1}$  as a standard, ranged from only 9% of the area by  $^{137}\text{Cs-USLE}$  up to the maximum, but still low, value of 16% by  $^{137}\text{Cs-WEPP}$ . The differences between  $^{137}\text{Cs}$  and WEPP and  $^{137}\text{Cs}$  and USLE followed a random or trend-less pattern. The differences between USLE and WEPP showed a clear trend, increasing from the upper slope reaching maximum values of  $>36 \text{ Mg ha}^{-1} \text{ yr}^{-1}$  from half-way down. The greater sensitivity to slope parameters and to crop-related factors in WEPP and its sediment-deposition estimates are clearly associated with these differences. Probably, if an internal relative soil-loss tolerance value were adopted, similar interpretations of erosion impacts would be achieved by WEPP and USLE at the upper slope and WEPP would attenuate erosion impacts at the mid and lower slopes.

The poor geographical coincidence is more evidence that the values resulting from non-calibrated soil-erosion methods should be considered as qualitative indications and that the target erosion-rate value has to be internally determined. In this case, the coherent geographic distribution, the local variability in relation to known sensitive method factors, the kind of output needed and the available database should be the key issues for method selection.

#### 4. CONCLUSIONS

The basic assumptions of the erosion-estimation method had a significant influence both on mean erosion or deposition rates and on geographic distribution patterns.

The election of the method to predict erosion can influence significantly the final interpretation of erosion-associated effects.

Method selection should consider overall site variability in relation to factors to which the methods are known to be sensitive.

#### REFERENCES

- [1] LAL, R., Soil erosion and land degradation: the global risks, *Adv. Soil Sci.* **7** (1990) 129–172.
- [2] LARSON, W.E., et al., (Eds.), *Proceedings of Soil Erosion and Productivity Workshop*, University of Minnesota, St. Paul (1990).
- [3] PIMENTEL, D., et al., Environmental and economic costs of soil erosion and conservation benefits, *Science* **267** (1995) 1117–1123.
- [4] CLARK E.H., et al., *Eroding Soils. The Off-Farm Impacts*, The Conservation Foundation, Washington, DC (1985).
- [5] LAL, R., “Soil erosion by wind and water: Problems and prospects”, *Soil Erosion Research Methods* (LAL, R., Ed.), Soil and Water Conservation Society, Ankeny, IA (1988).
- [6] EDWARDS, W.M., OWENS, L.B., Large storm effects on total soil erosion, *J. Soil Water Con.* **46** (1991) 75–78.

- [7] WISCHMEIER, W.H., SMITH, D.D., Predicting rainfall erosion losses—a guide to conservation planning, Agricultural Handbook 537, USDA, Washington, DC (1978).
- [8] FLANAGAN, D.C., NEARING, M.A., (Eds.), USDA-Water Erosion Prediction Project: Hillslope Profile and Watershed Model Documentation, West Lafayette: NSERL Report No. 10, USDA-ARS-WWA, West Lafayette, IN (1995).
- [9] RITCHIE, J.C., McHENRY, J.R., Application of radioactive fallout cesium-137 for measuring soil erosion and sediment accumulation rates and patterns: a review, *J. Env. Qual.* **19** (1990) 215–233.
- [10] WALLING, D.E., QUINE, T.A., Use of Caesium-137 as a Tracer of Erosion and Sedimentation: Handbook for the Application of the Caesium-137 Technique, UK Overseas Development Administration Research Scheme R4579, University of Exeter, Exeter (1993).
- [11] LANE, L.J., et al., Development and application of modern soil erosion prediction technology, *Aust. J. Soil Res.* **30** (1992) 893–912.
- [12] WALLING, D.E., HE, Q., Models for Converting <sup>137</sup>Cs Measurements to Estimate Soil Redistribution Rates on Cultivated and Uncultivated Soils. A contribution to the IAEA Coordinated Research Programmes on Soil Erosion (D1.50.05) and Sedimentation (F3.10.01), University of Exeter, Exeter (1997).
- [13] DENARDIN, J.E., Erodibilidade do Selo Estimada Por Meio de Parâmetros Físicos e Químicos, PhD Thesis, University of São Paulo, Piracicaba, Brazil (1990).
- [14] De MARIA, I.C. et al., “Fator C da Equação Universal de Perdas de Solo (EUPS) para a cultura de cana-de-açúcar”, Resumos da X Reunião Brasileira de Manejo e Conservação do Solo e da Água, Florianópolis, Summaries, Brazilian Society of Soil Science, Florianópolis (1994) 148–149.
- [15] RISSE, L.M., et al., Error assessment in the Universal Soil Loss Equation, *Soil Sci. Soc. Am. J.* **57** (1993) 825–833.
- [16] JOHNSON, R.R., Putting soil movement into perspective, *J. Prod. Agric.* **1** (1988) 5–12.
- [17] RENARD, K.G., (Coord.), Predicting Soil Erosion by Water: A Guide to Conservation Planning with the Revised Universal Soil Loss Equation (RUSLE), U.S. Department of Agriculture, Agricultural Handbook 703, USDA, Washington, DC (1997).
- [18] NEARING, M.A., et al., Sensitivity analysis of the WEPP hillslope profile erosion model, *Trans. ASAE* **33** (1990) 839–849.
- [19] McCORMACK, D.E., et al., “Current criteria for determining soil loss tolerance”, Determinants of Soil Loss Tolerance, *Am. Soc. Agron.*, Madison (1982) 95–111.

# USE OF THE $^{137}\text{Cs}$ TECHNIQUE IN SOIL-EROSION INVESTIGATIONS: A CASE STUDY IN THE ZITOUNA BASIN IN THE NORTH OF MOROCCO

M. BENMANSOUR, M. IBN MAJAH, H. MARAH, T. MARFAK  
Centre National de l'Energie des Sciences et des Techniques Nucléaires,  
Rabat, Morocco

D.E. WALLING  
Department of Geography,  
University of Exeter,  
Exeter, United Kingdom

## Abstract

Caesium-137 is a useful radionuclide for obtaining estimates of soil loss caused by erosion over a relatively long period of time (about 35 years). This artificial radionuclide was derived from atmospheric testing of nuclear weapons and was strongly adsorbed by fine soil particles. The measurement of the  $^{137}\text{Cs}$  inventory (in  $\text{Bq}\cdot\text{m}^{-2}$ ) allows rates of soil erosion and deposition to be determined. The  $^{137}\text{Cs}$  technique has been successfully applied in a wide range of environments and locations, particularly in the United Kingdom, the United States, and Australia. In this study, the technique was applied in Morocco to fields in the Zitouna basin located in the vicinity of El Hoceima. The results confirmed the potential for using the  $^{137}\text{Cs}$  technique in Morocco, but also identified some limitations and constraints due to local conditions.

## 1. INTRODUCTION

Soil erosion has serious effects on agriculture and the environment, chiefly:

- reduction in agricultural productivity as a consequence of decreased soil fertility and progressive loss of arable land, and
- downstream problems associated with increased sediment loads in rivers and sedimentation of reservoirs.

In Morocco, many drainage basins have been affected by erosion by water. It is estimated that about 20 Gha are under serious threat. The total loss of soil due to such erosion amounts to 100 Mt each year. Obtaining reliable data related to rates of erosion and redistribution of soil is very important in order to evaluate the severity of the problem and to optimize mitigating management practices. The classical methods of erosion assessment are based generally on the use of experimental plots or erosion modelling and have important limitations [1].

Using  $^{137}\text{Cs}$  as a tracer of soil allows calculation of erosion rate and the determination of soil movement in general. The first studies using the  $^{137}\text{Cs}$  technique were performed in the United States [2, 3], and the method has since been developed and exploited in a variety of environments [4–8]. However, its use in Morocco has been very limited [9, 10]. This paper gives a preliminary assessment of erosion in the Zitouna basin (El Hoceima region), which was selected with the collaboration of the Administration des Eaux et Forêts et de la Conservation des Sols.

## 2. BASIS OF THE $^{137}\text{Cs}$ TECHNIQUE

Caesium-137 is an artificial radionuclide that was released into the stratosphere by the testing of thermonuclear weapons from the 1950s to the 1970s. The radioactive fallout reached a peak around 1963, and levels of deposition were higher in the northern hemisphere than in the southern. The  $^{137}\text{Cs}$  that reached the soil surface, usually in association with precipitation, was rapidly and strongly adsorbed by the clay fraction [11, 12]. Under these conditions, the subsequent redistribution of the

$^{137}\text{Cs}$  radionuclide reflects the movement of the soil. Estimates of rates of erosion or deposition can be made by measurement of loss or gain in the radiocaesium inventory relative to a reference level. The main advantages of the  $^{137}\text{Cs}$  technique are [1]:

- It permits retrospective assessment of erosion rates.
- The redistribution rates represent an average for the past 35 years.
- It provides information of erosion rates and of spatial distribution.
- It requires only a limited number of field visits and results can be generated within a relatively short time.

Calibration models have been developed for quantitative estimation of erosion ( $\text{t ha}^{-1} \text{yr}^{-1}$ ). For cultivated soils, the proportional model [13–15] is most commonly used. It is assumed that  $^{137}\text{Cs}$  fallout inputs are completely mixed within the plough or cultivation layer and the soil loss is directly proportional to the reduction in the  $^{137}\text{Cs}$  of the soil profile. The proportional model can be represented as:

$$Y = 10 \times \frac{BdX}{100T}$$

where

- $X$  is percentage reduction in total  $^{137}\text{Cs}$  inventory =  $\frac{A - A_{ref}}{A_{ref}} \times 100$
- $Y$  is the mean annual soil loss ( $\text{t ha}^{-1} \text{yr}^{-1}$ ),
- $d$  is the depth of the plough or cultivation layer (m),
- $B$  is the bulk density of soil ( $\text{kg m}^{-3}$ ),
- $A_{ref}$  is the local  $^{137}\text{Cs}$  reference inventory ( $\text{Bq m}^{-2}$ ),
- $A$  is the measured total  $^{137}\text{Cs}$  inventory at the sampling point ( $\text{Bq m}^{-2}$ ),
- and  $T$  is the time elapsed since initiation of  $^{137}\text{Cs}$  accumulation (yr).

However, the proportional model does not take account of the dilution of  $^{137}\text{Cs}$  concentration in the soil within the plough layer due to incorporation of soil from below the original plough depth after surface lowering by erosion. The results obtained may underestimate the soil loss particularly where for erosion rates are high. The simplified mass balance [16,17] method, which assumes that the total  $^{137}\text{Cs}$  fallout occurred in 1963, is an improvement over the proportional model. This approach is expressed as:

$$Y = 10Bd \left[ 1 - \left( 1 - \frac{X}{100} \right)^{1/(t-1963)} \right]$$

where

- $t$  is the time since 1963.

The use of complex mass balance models [5, 15, 18–20] is more realistic, but additional information is needed. Empirical relationship models had been developed [21,22] also, but they are based on results from experimental plots. For undisturbed soils, in most situations, the depth distribution of  $^{137}\text{Cs}$  will exhibit an exponential decline with depth [15, 16]. It can be described as:

$$A'(x) = A_{ref}(1 - e^{-x/h})$$

where

- $x$  is the depth from the surface ( $\text{kg m}^{-2}$ ),
- $A'(x)$  is the amount of  $^{137}\text{Cs}$  above depth  $x$  ( $\text{Bq m}^{-2}$ ),
- $H$  is the coefficient describing the profile shape.

### 3. SITE CHARACTERISTICS AND SAMPLING STRATEGY

The field site was in the Zitouna basin (~6 km<sup>2</sup>) near Beni Boufrah, about 50 km west of El Hoceima. The altitude for the majority of fields ranges between 100 to 300 m and the mean annual precipitation is about 334 mm. A large part of the basin is reforested with cactus and pine. In the agricultural areas, cereals chiefly are grown.

The sampling strategy was based on the selection of fields (C1, C2, C3) on slopes of various steepness (Table I) and the identification of at least one suitable site in order to establish the reference inventory. To optimize the number of collected samples, the slope transect approach was adopted, which consists of a sequence of samples along the axis of greatest slope, from the upslope to the downslope boundary. The stoniness of the soil limited the frequency of sampling.

In addition, soil heterogeneity made it difficult to collect representative cores, therefore, two or three cores were collected at each sampling point. Samples were taken along one transect per field for C1 and C3 (T1, T3) and two transects were used on field C2 (C2-T1 and C2-T2).

Location of appropriate undisturbed reference sites [5] proved difficult. However, one reference site was identified for sampling.

A motorized cylindrical tube (ca. 67 cm<sup>2</sup>, 1 m in length) was used to collect samples. It was inserted to a depth of 30 cm in order to ensure that all <sup>137</sup>Cs was retained. The tube could be separated into two longitudinal sections to allow access to the sample. The whole soil core was collected when the <sup>137</sup>Cs inventory was required. When information about the depth distribution of <sup>137</sup>Cs was needed, incremental samples are collected at 1 or 2 cm. The positions of sampling points were determined by the GPS system.

### 4. LABORATORY ANALYSES

All samples were dried (~100°C for 24 h), lightly ground, sieved (<2mm) and mixed. A sub-sample of fine fraction was weighed into Marinelli beakers (~600 g) for whole soil cores or cylindrical containers (~100 g) for incremental samples. The <sup>137</sup>Cs measurements were made by gamma spectrometry using two high-purity coaxial germanium detectors (~30% efficiency, ~1.8 keV resolution). The <sup>137</sup>Cs activities were determined from the net area of the peak on the spectrum at 662 keV. The use of certified multi-gamma liquid solution and IAEA reference materials allowed the calibration of the detection-system efficiency for each counting geometry. The samples were counted for 12 to 24 h providing a precision of ca. 10% at the 90% level of confidence.

TABLE I. CHARACTERISTICS OF THE SELECTED FIELDS

Parameter	C1	C2	C3
Slope angle (%)	20	10	7
Slope length (m)	220	80	40
Altitude range (m)	190–260	130–150	120–130
Number of transects	1	2	1
Sampling point distance (m)	20	10	10

The  $^{137}\text{Cs}$  inventory ( $A$  in  $\text{Bq m}^{-2}$ ) can be expressed as:

$$A = C \cdot M/S$$

where

$C$  is the  $^{137}\text{Cs}$  activity of the sample ( $\text{Bq kg}^{-1}$ ),

$M$  is the total mass of the collected core,

and  $S$  is the cross-sectional area of the sampling tube.

## 5. RESULTS AND DISCUSSION

### 5.1. Distribution of $^{137}\text{Cs}$ within the soil profile

The field identified as a reference site was confirmed as such by the  $^{137}\text{Cs}$  profile (Fig. 1) which showed a sharp decline in  $^{137}\text{Cs}$  activity with most contained in the top 10 cm. Profiles for undisturbed sites have an exponential shape. The  $^{137}\text{Cs}$  concentration in the surface soil was about  $15 \text{ Bq kg}^{-1}$ .

For the cultivated soils,  $^{137}\text{Cs}$  radionuclide was almost uniform throughout the plough layer as a result of cultivation. The  $^{137}\text{Cs}$  profiles for the three selected fields (C1, C2, C3) are illustrated in Figs 2–4. The plough layer was not particularly deep, particularly on C1; the  $^{137}\text{Cs}$  was distributed within 12 to 14 cm of the surface. The concentration depends on the severity of soil loss. The studied cultivated sites had  $^{137}\text{Cs}$  concentrations at the surface of between 5 and  $15 \text{ Bq kg}^{-1}$ . These values are similar to those found previously in Morocco [24].

### 5.2. Caesium-137 inventories

The mean  $^{137}\text{Cs}$  value for the reference inventory, obtained from eight sampling points (R11–R18) was  $1,021 \pm 169 \text{ Bq m}^{-2}$  (Table II). With the exception of the third point (R13), which was high, there was no important deviation from the mean value. Generally, the variability in  $^{137}\text{Cs}$  inventories at a reference site has several sources (random spatial variability, systematic spatial variability, sampling variability, measurement precision) [25].

For the agricultural fields, the  $^{137}\text{Cs}$  inventories obtained for the sampling points collected along transects were generally lower than the reference inventory, particularly those on the upslope boundaries (C3 was a notable exception, see below) indicating the loss of soil. In contrast, high  $^{137}\text{Cs}$  inventories are generally observed at downslope boundaries, indicating deposition.

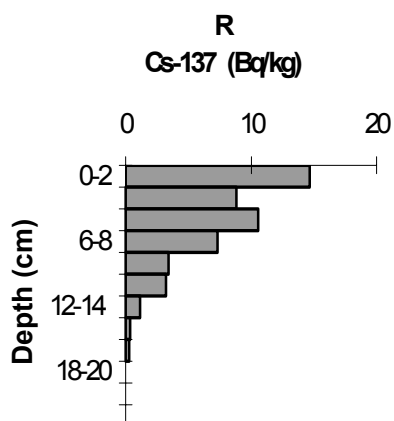


FIG. 1.  $^{137}\text{Cs}$  profile associated with the reference site (R).

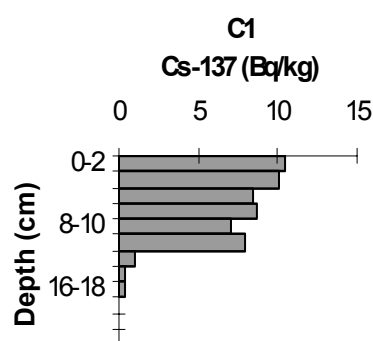


FIG. 2.  $^{137}\text{Cs}$  profile associated with cultivated site C1.

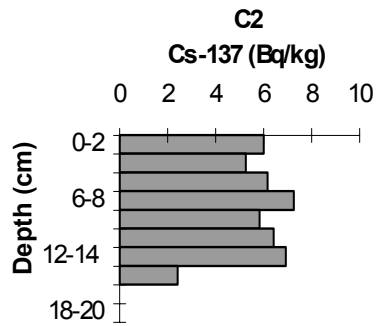


FIG. 3. <sup>137</sup>Cs profile associated with cultivated site C2.

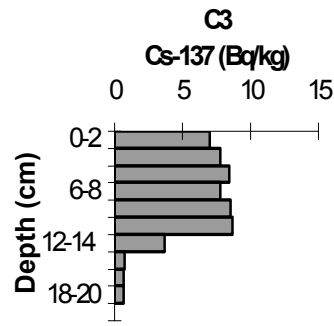


FIG. 4. <sup>137</sup>Cs profile associated with cultivated site. C3.

TABLE II. REFERENCE <sup>137</sup>Cs INVENTORY MEASUREMENTS

Sampling point	R11	R12	R13	R14	R15	R16	R17	R18
Activity (Bq m <sup>-2</sup> )	970	904	1,200	1,105	922	1,023	989	1,052

The <sup>137</sup>Cs inventories in the eroding zones ranged between 300 and 800, 400 and 1,000, and 600 and 900 Bq m<sup>-2</sup> for the C1, C2, and C3 fields, respectively. The values were distinctly lower than the reference inventory. However, the first sampling points at the top of field C3 were higher (~1,800 Bq m<sup>-2</sup>) than the reference inventory.

### 5.3. Estimation of erosion and deposition rates

The rates of soil erosion and deposition were determined by applying the proportional and simplified mass balance models, as shown in Figs 5 to 8, in which erosion is denoted by a minus sign. Differences between the two models were most marked where rates of erosion and deposition were highest. The mass balance model (as described in Section 2) was used for the final interpretation of the results.

In field C1, with a slope of ~20% and 220 m in length, the erosion zone represented 83% of the total length and only 17% of the transect length was a deposition zone (Fig. 5). Erosion rates varied from 12 to 40 t ha<sup>-1</sup> yr<sup>-1</sup>, reflecting complex topography. The slope angle was not uniform along the transect and may explain differences in rate of erosion from one sampling point to another and also the deposition of soil before reaching the downslope boundary. Furthermore, the three last sampling points were found to be part of an eroded area, probably due to the increase in the slope. Taking into account the total length of the transect, the gross erosion rate was approximately 18.6 t ha<sup>-1</sup> yr<sup>-1</sup> and the gross deposition rate was 2.0 t ha<sup>-1</sup> yr<sup>-1</sup>. The net erosion rate that represents the amount of soil leaving the field was 16.6 t ha<sup>-1</sup> yr<sup>-1</sup> corresponding to a 76% sediment delivery ratio.

For field C2 (slope ~10%), the soil movements in transects C2-T1 and C2-T2 (Figs 6 and 7) were approximately the same, at least for eroding zones, confirming the validity of the lateral uniform <sup>137</sup>Cs distribution hypothesis and the slope-transect approach. Nevertheless, C2-T1 transect showed more soil deposition than C2-T2. The gross erosion rate (mean from the two transects) was about 11.9 t ha<sup>-1</sup> yr<sup>-1</sup>, lower than the value obtained for field C1 with a greater slope. The net erosion rate was 5.4 t ha<sup>-1</sup> yr<sup>-1</sup>, which corresponds to a 50% ratio of sediment delivered.

Concerning field C3 (slope  $\sim 7\%$ ), the high  $^{137}\text{Cs}$  inventories at the top compared to the reference inventory indicates sedimentation from a hillock located at 40 m distance. Estimating the erosion rate for the total length of the field was difficult. However, by ignoring the first sampling points, the data are more meaningful. Soil redistribution in C3 is illustrated in the Fig. 8. The values for gross and net soil erosion rates were  $7.2$  and  $6.3 \text{ t}\cdot\text{ha}^{-1}\cdot\text{yr}^{-1}$ , respectively, corresponding to the delivery of the totality of eroded soil ( $\sim 90\%$ ) from this cultivated site.

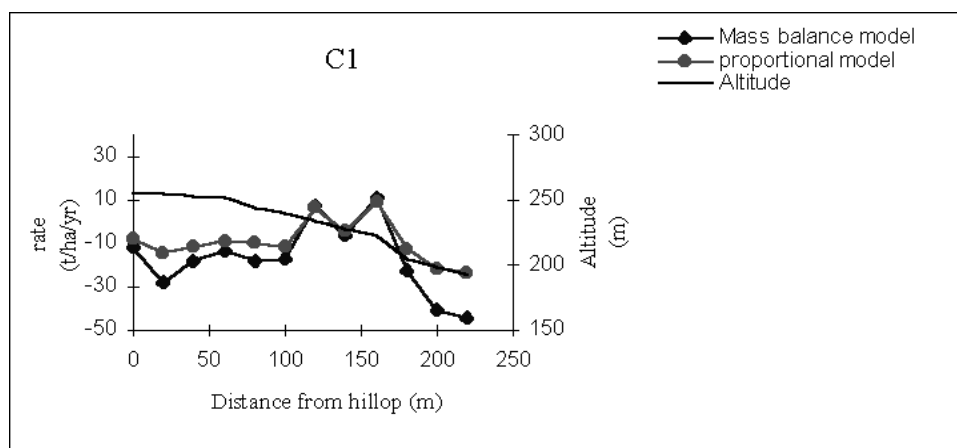


FIG. 5. Soil redistribution rate in field C1.

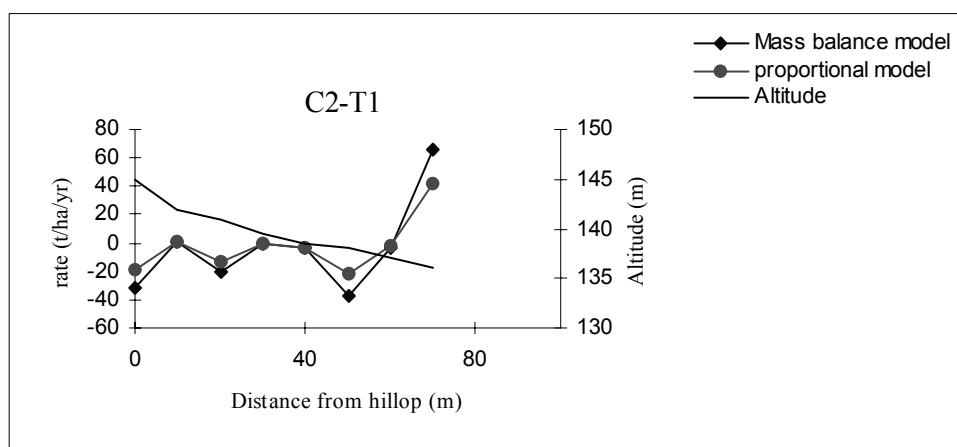


FIG. 6. Soil redistribution rate in field C2 (transect C2-T1).

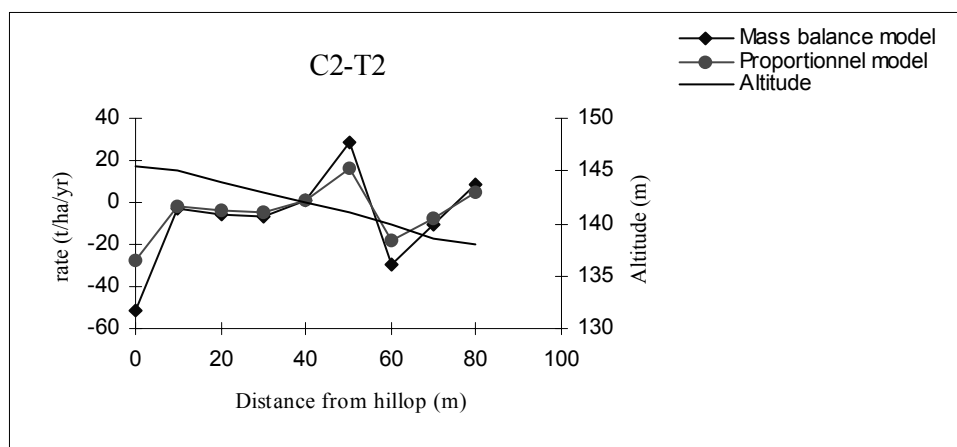


FIG. 7. Soil redistribution rate in field C2 (transect C2-T2).



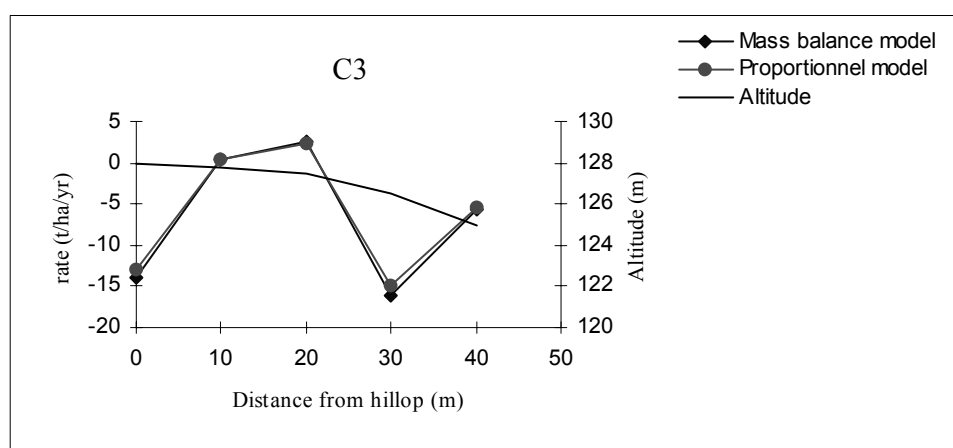


FIG.8. Soil redistribution rate in field C3.

TABLE III. INTEGRATED EROSION- AND DEPOSITION-RATE DATA FOR THE THREE CULTIVATED SITES

Field	Erosion rate (t ha <sup>-1</sup> yr <sup>-1</sup> )		Deposition rate (t ha <sup>-1</sup> yr <sup>-1</sup> )		Net erosion rate (t ha <sup>-1</sup> yr <sup>-1</sup> )	Sediment delivery (%)
	Mean	Gross	Mean	Gross		
C1	22.3	18.6	11.7	2.0	16.6	77
C2 (C2-T1)	19.2	12.0	22.9	8.8	3.4	28
C2 (C2-T2)	17.6	11.8	12.9	4.3	7.4	77
C3	11.9	7.2	2.1	0.8	6.3	88

The results for the three fields are summarized in Table III. Although the soil-erosion rates were based on the simplified mass balance model, which takes account of loss of soil by water erosion only, effects of tillage [18–20] may explain the high erosion rates obtained for the first sampling points, particularly on field C2.

## 6. CONCLUSION

The Zitouna basin case study demonstrated the potential for using the <sup>137</sup>Cs technique in Morocco to provide erosion- and deposition-rate data. The main problems encountered were the identification of appropriate undisturbed reference sites and stoniness associated with soil heterogeneity for the cultivated sites. Although the sampling points were limited, the results provided preliminary indications regarding soil erosion and sediment delivery. Because of the severity of erosion in Morocco, the <sup>137</sup>Cs technique appears to be suitable for estimating soil loss, complementing the classical techniques used in national agricultural programs.

## REFERENCES

- [1] WALLING, D.E., QUINE, T.A., “Use of fallout radionuclide measurements in soil erosion investigations”, Nuclear and Related Techniques in Soil-Plant Studies on Sustainable Agriculture and Environment Preservation, IAEA, Vienna (1995) 597–619.
- [2] ROGOWSKI, A.S., TAMURA, T., Erosional behavior of <sup>137</sup>Cs, Health Phys. **18** (1970) 467–477.

- [3] RITCHIE, J.C., et al., Estimating soil erosion from the distribution of fallout  $^{137}\text{Cs}$ , *Soil Sci. Soc. Am. Proc.* **38** (1974) 137–139.
- [4] QUINE, T.A., WALLING, D.E., Rates of Soil Erosion on Arable Fields in Britain: Quantitative data from caesium-137 measurements, *Soil Use Management* **7** (1991) 169–176.
- [5] WALLING, D.E., QUINE, T.A., Use of  $^{137}\text{Cs}$  as a Tracer of Erosion and Sedimentation, *Handbook for the Application of the  $^{137}\text{Cs}$  Technique*, University of Exeter, Exeter (1993).
- [6] RITCHIE, J.C., MCHENRY, J.R., Application of radioactive fallout  $^{137}\text{Cs}$  for measuring soil erosion and sediment accumulation rates and patterns: a review, *J. Environ. Qual.* **19** (1990) 215–233.
- [7] ELLIOT, G.L., et al., Correlation of erosion measurements and soil  $^{137}\text{Cs}$  contents, *Appl. Rad. Isot.* **39** (1990) 1153–1157.
- [8] BERNARD, C., LAVERDIÈRE, M.R., Variation spatiale de l'activité en  $^{137}\text{Cs}$  et ses répercussions sur l'estimation de l'érosion hydrique, *Pédologie* **40** (1990) 299–310.
- [9] BOUHLASSA, S., et al.,  $^{137}\text{Cs}$  fallout as tracer of erosion and sedimentation in big catchment, *App. Radiat. Isot.* **46** (1994).
- [10] LAHLOU, Y., Erosion dans le Bassin Versant du Barrage Sidi Driss: Méthodologie d'Approche et Quantification (Cas du sous Bassin Versant Mhasser), Thèse de 3<sup>ème</sup> Cycle, Université Mohamed, Faculté des Sciences de Rabat, Maroc (1997).
- [11] LIVENS, F.R., BAXTER, M.S., Chemical associations of artificial radionuclides in Cumbrian soils, *Environ. Radioact.* **7** (1988) 75–86.
- [12] BACHHUBER, H., et al., The migration of  $^{137}\text{Cs}$  and  $^{90}\text{Sr}$  in multilayered soil minerals, results from batch, column and fallout investigations, *Nucl. Technol.* **59** (1982) 291–301.
- [13] MITCHELL, J.K., et al., "Soil loss estimation from  $^{137}\text{Cs}$  measurements", *Assessment of Erosion* (DeBoodt, M., Gabriels, G., Eds.), Wiley, Chichester (1980) 393–401.
- [14] MARTZ, L.W., DE JONG, E., Using  $^{137}\text{Cs}$  to assess the variability of net soil erosion and its association with topography in a Canadian prairie landscape, *Catena* **14** (1987) 439–451.
- [15] WALLING, D.E., QUINE, T.A., Calibration of  $^{137}\text{Cs}$  measurements to provide quantitative erosion rate data, *Land Degrad. Rehabil.* **2** (1990) 161–175.
- [16] ZHANG, X.B., et al., A preliminary assessment of the potential for using  $^{137}\text{Cs}$  to estimate rates of soil erosion in the Loess Plateau of China, *Hydrol. Sci. J.* **35** (1990) 267–276.
- [17] KACHANOSKI, R.G., Estimating soil loss from changes in soil caesium-137, *Can. J. Soil. Sci.* **73** (1993) 715–526.
- [18] HE, Q., WALLING, D.E., The distribution of fallout  $^{137}\text{Cs}$  and  $^{210}\text{Pb}$  in undisturbed and cultivated soils, *App. Radiat. Isot.* **48** (1997) 677–690.
- [19] GOVERS, G., et al., The relative contribution of soil tillage and overland flow erosion to soil redistribution on agricultural land, *Earth Surf. Proc. Landforms* **21** (1996) 929–946.
- [20] QUINE, T.A., et al., "Simulation of radiocaesium redistribution on cultivated hillslopes using a mass-balance model: an aid to process interpretation and erosion rates estimation", *Advances in Hillslope Process* (ANDERSON, M.G., BROOKS, S.M., Eds.), Wiley, Chichester (1996) 561–588.
- [21] RITCHIE, J.C., MCHENRY, J.R., Fallout  $^{137}\text{Cs}$ : a tool in conservation research, *J. Soil Water Conserv.* **30** (1975) 283–286.
- [22] CAMPBELL, B.L., et al., "Mapping drainage basin sources using caesium-137", *Drainage Basin Sediment Delivery*, (HADLEY, R.F., Ed.), IAHS, Wallingford (1986) 437–446.
- [23] LOUGHRAN, R.J., et al., Estimation of soil erosion from caesium-137 measurements in a small cultivated catchment in Australia, *Appl. Radiat. Isot.* **39** (1988) 1153–1157.
- [24] AIT HADDOU, A., et al., Evaluation of  $^{137}\text{Cs}$  "Pollution in Morocco", *International Symposium on Environmental and Impact Assessment*, Mohammedia, October 9–11, (1996).
- [25] OWENS, P.N., WALLING, D.E., Spatial variability of  $^{137}\text{Cs}$  inventories at reference sites: an example from two contrasting sites in England and Zimbabwe, *App. Radiat. Isot.* **47** (1996) 699–707.

## VARIABILITY OF $^{137}\text{Cs}$ INVENTORIES IN UNDISTURBED SOILS ACROSS THE TERRITORY OF VIET NAM

P.D. HIEN, H.T. HIEP, N.H. QUANG, N.Q. HUY,  
N.T. BINH, P.S. HAI, N.Q. LONG, V.T. BAC

Viet Nam Atomic Energy Agency,  
Hanoi, Viet Nam

### Abstract

Inventories of  $^{137}\text{Cs}$  were measured in putatively undisturbed soils at 292 locations throughout the territory of Viet Nam. Logarithms of these values were regressed against characteristics of the sampling sites, such as geographical co-ordinates, annual rainfall and physico-chemical parameters of soil. The regression model containing latitude and annual rainfall, as explanatory variables, could explain 76% of the variation in logarithmic inventory values. The model was interpreted to represent the spatial distribution of  $^{137}\text{Cs}$  deposition density while the regression residuals were assigned to the loss or gain of  $^{137}\text{Cs}$  due to soil erosion or accretion at the sampling sites. Depth-distribution profiles of  $^{137}\text{Cs}$ , measured at eight selected sites, provided support for this interpretation. In particular, a linear relationship was found between the residual and the  $^{137}\text{Cs}$ -penetration depth. Although, on average, the measured inventories differed from the deposition values by 31%, the  $^{137}\text{Cs}$  deposition density could be predicted by the regression model with a  $\pm 7\%$  relative uncertainty at a 95% confidence level. The model has, therefore, been used to provide  $^{137}\text{Cs}$  baseline values in soil-erosion studies. These results are in general agreement with the global pattern published in 1969 by the United Nations Scientific Committee on the Effects of Atomic Radiation, and provide further insights into the spatial distribution of nuclear-test fallout deposition in East Asia.

### 1. INTRODUCTION

Fallout  $^{137}\text{Cs}$  from past atmospheric nuclear tests has been successfully used as a tracer for studies of soil-erosion and sedimentation [1–3]. So far, most have been carried out in mid-latitude regions [4], where maximum fallout deposition was observed in both hemispheres [5]. For tropical countries, only a few reports have appeared (e.g. Refs [6, 7]). Among the reasons may be the low  $^{137}\text{Cs}$  inputs, especially in areas near the equator, where global fallout deposition was minimal. Moreover, fallout data are scarce for large areas of the tropics, making uncertain the prediction of the  $^{137}\text{Cs}$  baseline inputs in designing erosion studies.

The level of  $^{137}\text{Cs}$  deposition in Viet Nam—a 320,000 km<sup>2</sup> territory extending from about 9°N to 23°N along the west coast of the Pacific Ocean (Fig. 1)—is expected to increase northward from about 300 to 600 Bq m<sup>-2</sup>, according to the global fallout deposition pattern reported by the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) [5]. The contribution from the Chernobyl accident in 1986 was insignificant based on the results of fallout radioactivity measurements carried out in Dalat, South Viet Nam, from 1986 to 1990 [8]. In Ref. [7] a mean  $^{137}\text{Cs}$  inventory of (330±67) Bq m<sup>-2</sup> was found in undisturbed soils of some drainage basins in South Viet Nam (≈12°N, 108°E), consistent with the above lower limit, some 7 to 15% of values seen in European countries (e.g. Refs. [2,3]). In contrast, surveys of surface soils in North Viet Nam showed areas with inventory values much higher than the above upper limit of 600 Bq m<sup>-2</sup> [9].

In view of this situation, the measurement of  $^{137}\text{Cs}$  inventories in undisturbed soils was necessary for the planning of erosion studies. The project, executed from 1997 to 1999, yielded inventory values for 292 sites. Multiple regression of these data upon possibly relevant characteristics of the sites, such as latitude, annual rainfall and physico-chemical parameters of the soil, allowed the construction of models describing the spatial distributions of  $^{137}\text{Cs}$  deposition density over the whole territory.

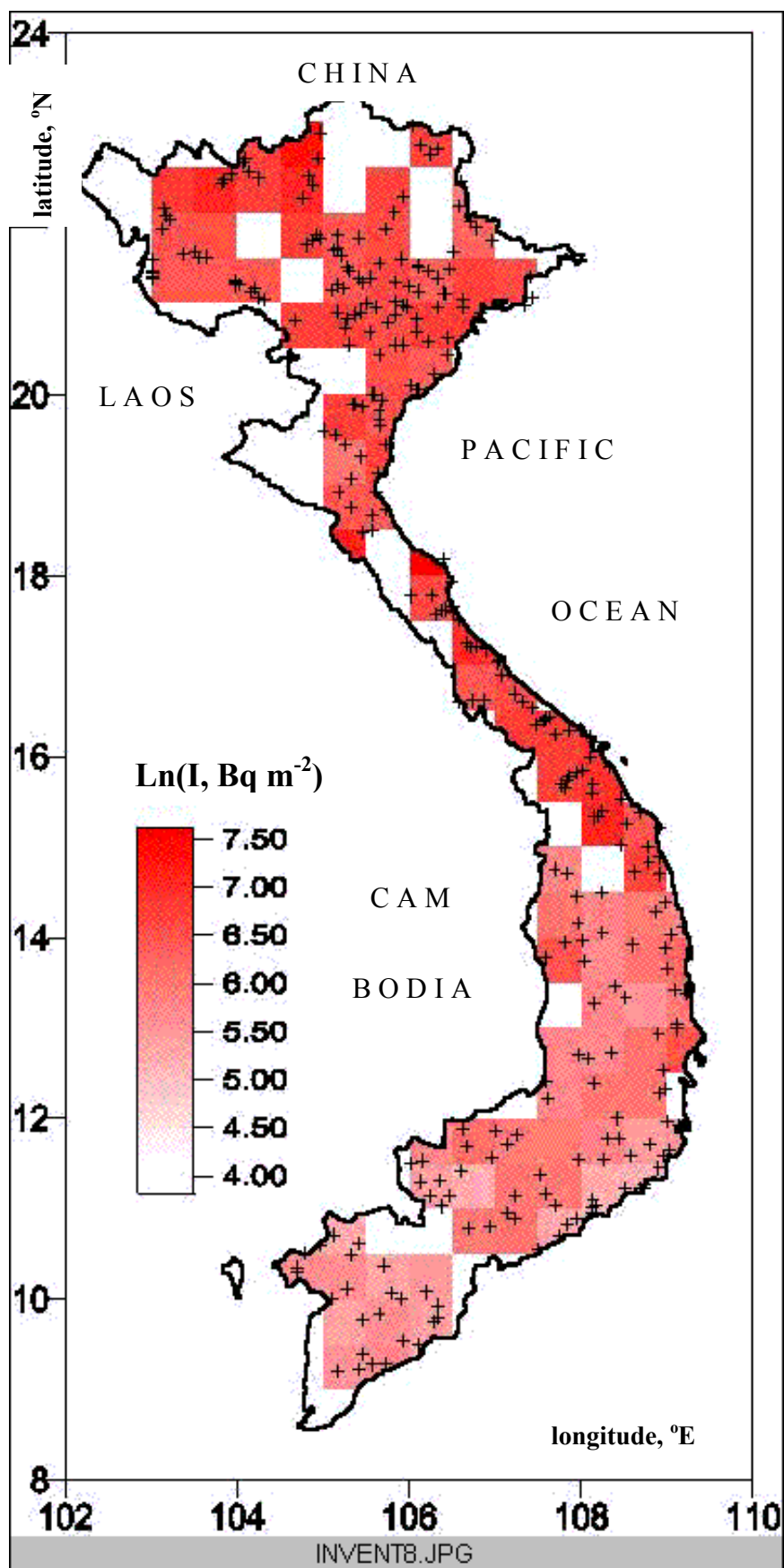


FIG. 1. Sampling map and spatial distribution of  $^{137}\text{Cs}$  inventories.

## 2. MATERIALS AND METHODS

### 2.1. Soil sampling

Each soil sample was taken to a depth of 30 cm on flat, grass-covered terrain believed to have been undisturbed for four decades. A total of 292 samples were collected from ten geographical/climatic regions. Sites selected included virgin land, forest preserves, orchards, meteorological gardens, historical places, etc. Mountainous and remote areas were not included. On the grid (0.5°×0.5°), one hundred of 140 grid cells were sampled (Fig. 1), in which the average number of samples per grid cell was 2.9±1.6.

About 80% of the soils collected are acrisols, fluvisols and ferralsols, the three major groups, which account for 85% of Viet Nam's territory [10].

### 2.2. Analyses

Prior to gamma-spectrometry measurements, the materials were oven-dried (105°C, 24 h), gently ground to powder and sieved to pass through a 2-mm mesh. The bulk density of each soil was determined by weighing. Activity concentrations of radionuclides were measured at five low-background gamma-spectroscopy laboratories using high-purity Ge detectors with active volumes of 90 to 140 cm<sup>3</sup> and peak resolutions (FWHM) of around 1.5 keV at 662 keV. The minimum detectable activity defined as three background areas at the 662 keV photopeak was around 0.3 Bq kg<sup>-1</sup> for a 24-h counting time. The precision of gamma-spectrometry measurements calculated as one standard error of the net area of the <sup>137</sup>Cs 662 keV photopeak was from 7% to 15%.

Physico-chemical properties of the soils, including pH, organic matter content, cation-exchange capacity, granulometric composition, etc., were determined according to FAO guidelines [11]. The measured <sup>137</sup>Cs concentrations and inventories, as well as relevant characteristics of sampling sites, are summarized in Table I.

## 3. REGRESSION ANALYSIS

### 3.1. Regression model

The stepwise method of multiple linear regression was applied to identify characteristics of the sampling sites that are most relevant in explaining the variations of <sup>137</sup>Cs inventories, and to establish the relationships statistically. This technique was used in a previous study of <sup>137</sup>Cs fallout in a Texas watershed ecosystem [12]. In this work, the logarithmic <sup>137</sup>Cs inventories were used as a dependent variable. Such a logarithmic transformation allowed us to obtain regression models with high goodness-of-fit (high R<sup>2</sup>-values). The physical interpretation of such a logarithmic transformation is discussed later.

The stepwise linear regression method selected among characteristics of sampling locations (Table I) explanatory variables according to the criteria set for significance level of the regression coefficients. If this level was set at 0.05, only latitude L (°N) and annual rainfall AR (m) appeared as explanatory variables:

$$\begin{aligned} \text{Ln}(I, \text{Bq m}^{-2}) - \varepsilon &= (3.53 \pm 0.09) + (0.092 \pm 0.004)L + (0.62 \pm 0.03)AR \\ (1) \qquad \qquad \qquad [t = 37.9] \qquad \qquad [22.8] \qquad \qquad [19.9] \end{aligned}$$

TABLE I. SUMMARY STATISTICS OF MEASURED DATA (292 SAMPLES)

	Mean	Std. dev.	Median	Range
Latitude (°N)				9.2 – 23
Longitude (°E)				103 – 110
<sup>137</sup> Cs activity conc. (Bq kg <sup>-1</sup> )	2.03	2.20	1.58	0.5 – 18.0
<sup>137</sup> Cs inventory (Bq m <sup>-2</sup> )	643	384	590	129 – 3,294
Bulk density (kg m <sup>-3</sup> )	1.22	0.30	1.23	0.43 – 2.1
Mean annual rainfall (m)	1.97	0.56	1.88	0.79 – 3.8
Content of soil organic matter (%)	2.8	1.7	2.5	0.3 – 8.5
Clay + silt (%)	81	13	80	29 – 87
pH	4.8	1.1	4.4	3.4 – 7.9

Very high t-values shown in the square brackets indicate the significance of the statistical relationship (1). The two variables L and AR explain 43% and 33% ( $R^2 = 0.76$ ) of the total variance of  $\text{Ln}(I)$ , respectively, leaving the 24% remaining variance to the residual  $\varepsilon$ . The latter follows an approximately normal distribution with zero mean ( $\bar{\varepsilon} = 0$ ) and a standard deviation of 0.30, which is apparently greater than that associated with experimental errors. The latter can be estimated as  $\text{Ln}(1 + \alpha) \approx 0.1$ , where  $\alpha$  is the typical relative standard error of the inventory measurements ( $\alpha \approx 10\%$ ). Physico-chemical parameters of soil listed in Table I did not contribute to explaining the variations in  $\varepsilon$ . However, if the regression involved samples taken from any particular geographical region with insignificant variation in latitude and annual rainfall, then soil parameters appear to partly explain the variations in the inventory values.

### 3.2. Interpretation of model (1)

If all the samples were actually taken from undisturbed sites, the inventory measurement would yield the <sup>137</sup>Cs deposition density (D) pattern, which is controlled by the latitude (L) and annual rainfall (AR). In this case, the multiple linear regression of  $\text{Ln}(I)$  upon characteristics of sampling sites would result in model (1), in which the right-hand side represents  $\text{Ln}(D)$  and the residuals are governed by the experimental errors,  $\varepsilon_{\text{exp}} \approx \text{Ln}(1 + \alpha) \approx 0.1$ . We have in this case:

$$\text{Ln}(I) - \varepsilon_{\text{exp}} = \text{Ln}(D(L, AR)) \quad (2)$$

As a matter of fact,  $|\varepsilon|$ 's are apparently greater than  $\varepsilon_{\text{exp}}$ , indicating that most samples were not taken from truly undisturbed sites. In practice, redistribution processes involving erosion, transport, and accretion of surface soil have occurred, causing either loss or gain of <sup>137</sup>Cs at the sampling locations. Hence measured inventory I was either smaller or greater than the deposition density D. It is natural, however, to assume that both cases were equally likely to occur in our survey, so that the mean logarithmic inventory for sampling locations having the same L and AR tends to converge toward the logarithmic deposition density  $\text{Ln}(D)$ . The regression of inventory values yields, therefore, the relationship between I and D:

$$\text{Ln}(I) - \varepsilon = \text{Ln}(D(L, AR)) \quad (1')$$

where

$\varepsilon$  is a measure of surface soil erosion or accretion, i.e.  $\varepsilon = 0$  for undisturbed sites and  $\varepsilon < 0$  and  $\varepsilon > 0$  for erosional and accretional sites, respectively.

TABLE II. CHARACTERISTICS OF SITES SELECTED FOR  $^{137}\text{Cs}$  DEPTH DISTRIBUTION PROFILE MEASUREMENTS

Site	L (°N)	AR (m)	Organic matter (%)	Silt + clay (%)	Vegetation	I (Bq m <sup>-2</sup> )	$\varepsilon$	d <sub>0.8</sub> (cm)
PT-11	21.47	1.85	1.01	47	grass	328±50	-0.75	7.5
CB-10	22.65	1.37	1.5	74	grass	375±40	-0.55	8
HB-1	20.90	1.60	2.2	70	grass	680±70	-0.08	11.7
DT-5	11.59	1.67	2.8	55	grass	258±30	-0.07	9.5
HT-11	17.94	2.60	2.7	40	grass	857±70	0	10.5
DN-2	11.53	1.57	3.1	51	grass	306±30	0.13	9
DN-1	11.51	1.57	3.5	60	grass	308±30	0.16	11
BT-17	11.04	1.70	1.5	72	grass/forest	340±28	0.22	12.5
HCM-2	10.79	1.93	2.5	20	grass/forest	461±40	0.40	21

The mean deviation between inventory and deposition density was 0.3 and 31% in logarithmic and ordinary scales, respectively. However, as the regression analysis involved a large number of samples, the deposition density could be predicted from the latitude and annual rainfall for any location using regression model (1) with much greater certainty, namely at about 7% at a 95% confidence level ( $\pm 2\sigma$ ).

### 3.3. $^{137}\text{Cs}$ depth distribution profiles

The  $^{137}\text{Cs}$  depth distribution profiles, which are sensitive to surface-soil disturbance [2, 6, 13, 14] would provide support for the above interpretation. Nine sites were selected for the study of the  $^{137}\text{Cs}$  depth-distribution profiles. The residuals  $\varepsilon$  and relevant characteristics of the sites are listed in Table II. The penetration depth of  $^{137}\text{Cs}$ , which was estimated as the thickness of the top layer containing 80% of the total inventory ( $d_{0.8}$ ), is presented in Table II. In most cases, the  $^{137}\text{Cs}$  concentration exhibited a peak followed by an approximately exponential decline with depth. The penetration depths generally increased with  $\varepsilon$  resulting in a high correlation ( $R = 0.85$ ) between  $\varepsilon$  and  $\text{Ln}(d_{0.8})$ . For cases with  $\varepsilon \approx 0$  the penetration depth  $d_{0.8}$  was from 9 to 12 cm. These features are similar to those obtained by other researchers [e.g. 2, 3, 13–15] for non-eroded uncultivated soils. The penetration depths were apparently greater than the above range for  $\varepsilon > 0.2$  and around 7 to 8 cm for the two cases with  $\varepsilon < -0.5$ . Therefore, the depth distribution measurements fully justified the above interpretation of regression model (1). In particular, the zero residuals ( $\varepsilon \approx 0$ ) corresponded to truly undisturbed sites. As an implication, the right-hand side of (1) represents the spatial distribution of  $^{137}\text{Cs}$  deposition density across the territory.

## 4. SPATIAL DISTRIBUTION OF $^{137}\text{Cs}$ DEPOSITION DENSITY

As would be expected from the above finding, the effects of  $^{137}\text{Cs}$  redistribution—erosion from some sites and deposition at others—would be smoothed out if the averages of inventory values were taken over adjacent sampling points. For example, if  $\text{Ln}(I)$  and other characteristics of the sampling locations were averaged over ( $0.5^\circ \times 0.5^\circ$ ) grid cells (Fig. 1), the regression model for the averages would be:

$$[\text{Ln}(I, \text{Bq m}^{-2})] - \varepsilon' = (3.51 \pm 0.11) + (0.093 \pm 0.005)[L] + (0.61 \pm 0.04)[AR] \quad (3)$$

[t = 33.1]                      [20.6]                      [12.1]

The intercept and regression coefficients in (3) remain similar to those in (1), indicating that the right-hand side of (3) represents the grid-cell average logarithmic deposition density [Ln(D)]. The new regression model could explain 88% of the variance in [Ln(I)] taken at one hundred grid cells over the territory ( $R^2 = 0.88$ ). Accordingly, the deviations between [Ln(I)] and [Ln(D)] are very small, and the standard deviation of the new residuals  $\varepsilon'$  was 0.17, much less than that of the original model (1). The spatial distribution of [Ln(I)] is mapped in Fig. 1, which clearly shows the effect of the annual rainfall variability superimposed on the northward increasing trend in deposition density. The results obtained in this work are generally in agreement with global data in UNSCEAR's 1969 report [5], and provide further insights in the spatial distribution pattern of nuclear test fallout deposition in East Asia.

## 5. DISCUSSION AND CONCLUSIONS

Two factors controlling the spatial variability of the deposition rate of  $^{137}\text{Cs}$  were its atmospheric concentration and the amount of rainfall during the period of thermonuclear-test fallout. The product relationship between deposition rate and these two factors results in a linear relationship of the logarithmic deposition density with the latitude and annual rainfall, as revealed by regression model (1). The model could explain 76% of the total variance in the logarithmic inventories measured for 292 sampling sites across the territory. The remaining variance contained in the regression residuals mainly represents the deviations of the measured inventories from the deposition density due to erosion or accretion at sampling sites. Thus, the regression model yielded the deposition-density pattern across the territory, while the regression residual  $\varepsilon$  provided a measure of the extent of erosion ( $\varepsilon < 0$ ) or accretion ( $\varepsilon > 0$ ) at each sampling site. The above interpretation was confirmed by the measurements of  $^{137}\text{Cs}$  depth-distribution profiles.

Despite efforts to find undisturbed soils for inventory measurements, most of the sites chosen were shown to have undergone either erosion or accretion. The average deviation between the measured inventory and the deposition density was about 31%. However, due to the large number of samples studied and because erosional and accretional sites were equally likely to occur in the sampling campaign, the  $^{137}\text{Cs}$ -deposition density at any location could be predicted with a much higher certainty, namely within about  $\pm 7\%$  at the 95% confidence level. The results of this work have successfully been applied to providing  $^{137}\text{Cs}$  baseline values for studies of erosion.

## ACKNOWLEDGEMENTS

This study was sponsored by the Ministry of Planning and Investment, and was performed under supporting administrative arrangements with the Ministry of Science Technology and Environment. A great number of colleagues of Viet Nam Atomic Energy Agency participating in sample collection and preparation are gratefully acknowledged. This work was also partly funded by an IAEA Technical Co-operation Programme.

## REFERENCES

- [1] RITCHIE, J.C., McHENRY, J.R., Fallout  $^{137}\text{Cs}$ : A tool in conservation research, *J. Soil Water Cons.* **30** (1975) 283–286.
- [2] WALLING, D.E., QUINE, T.A., The use of  $^{137}\text{Cs}$  measurements to investigate soil erosion on arable fields in the UK: potential applications and limitations, *J Soil Sci.* **42** (1991) 147–165.
- [3] LOUGHRAN R.J., et al. Soil erosion and sedimentation indicated by  $^{137}\text{Cs}$ : Jackmoor Brook catchment, Devon, England, *Catena* **14** (1987) 201–212.
- [4] INTERNATIONAL ATOMIC ENERGY AGENCY, Use of  $^{137}\text{Cs}$  in the Study of Soil Erosion and Sedimentation, IAEA-TECDOC-1028, Vienna (1998).
- [5] UNITED NATIONS, United Nations Scientific Committee on the Effects of Atomic Radiation 1969, 24th Session, Suppl. No 13 (A/7613), United Nations, New York (1969).
- [6] GARCIA-OLIVA, F., et al., Long-term net soil erosion as determined by  $^{137}\text{Cs}$  redistribution in an undisturbed and perturbed tropical deciduous forest ecosystem, *Geoderma* **68** (1995) 135–147.



- [7] HAI, P.S., et al. "Assessment of erosion and accretion in catchment areas based on  $^{210}\text{Pb}$  and  $^{137}\text{Cs}$  contents in soil and sediment," Proc. 3rd Int. Conf. on Isotopes, Vancouver, Canada (STEVENSON, N.R., Ed.), World Scientific, London (1999).
- [8] HIEN, P.D., et al. Variations of caesium isotope concentrations in air and fallout at Dalat, south Viet Nam, 1986-91, J. Environ. Radioactivity **22** (1994) 55–62.
- [9] VIET NAM ATOMIC ENERGY COMMISSION, Reports on the Implementation of Scientific Programmes (1991, 1998) (in Vietnamese).
- [10] VIET NAM SOIL SCIENCE ASSOCIATION, Soils of Viet Nam. Agriculture Publishing House, Hanoi (1996).
- [11] FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS, Procedures for Soil Analysis (Fifth Edition), FAO, Wageningen (1995).
- [12] McHENRY, J.R., RITCHIE, J.C., Physical and chemical parameters affecting transport of  $^{137}\text{Cs}$  in arid watersheds, Water Resource Res. **13** (1977) 923–927.
- [13] OWENS, P.N., et al., The behavior of bomb-derived  $^{137}\text{Cs}$  fallout in catchment soils, J. Environ. Radioactivity **32** (1996) 169–191.
- [14] TURNAGE, K.M., et al., Comparison of soil erosion and deposition rates using radiocesium, RUSLE and buried soils in dolines in East Tennessee, Environ. Geology **29** (1997) 1–10.
- [15] HILLMANN, U., et al., In situ  $\gamma$ -spectrometry several years after deposition of radiocesium, Radiat. Environ. Biophys. **35** (1996) 297–303.

**RECENT ADVANCEMENTS IN ISOTOPE ANALYTICAL  
METHODOLOGIES AND RELATED INSTRUMENTATION**

(Session 7)



**Keynote Address****ADVANCES AND FUTURE TRENDS IN  
RADIOISOTOPE ANALYSIS AND APPLICATIONS**

M.F. L'ANNUNZIATA

The Montague Group,  
Oceanside, California, United States of America**Abstract**

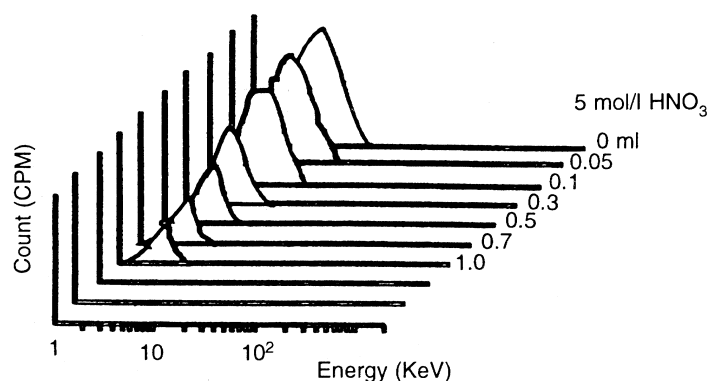
Modern advances that facilitate the accurate activity analysis of radionuclides as either single or multiple isotope tracers are described, with a focus on applications in the agricultural and biological sciences. The advances comprise both instrumental design and techniques. These are described in the following topics: (i) liquid scintillation analysis (LSA) via a quench-indicating parameter (QIP), (ii) direct DPM methods, (iii) low-level liquid scintillation analysis, (iv) Cherenkov counting, (v) microplate scintillation analysis, (vi) multiple radionuclide analysis, (vii) solid scintillation analysis, (viii) radionuclide standardization, (ix) instrument performance assessment (IPA), (x) 'Replay', and (xi) flow scintillation analysis (FSA). The use of LSA with a QIP will remain the mainstay analytical technique for years to come. Modern liquid scintillation analysers equipped with bismuth germanate detector guards and time-resolved liquid scintillation counting (TR-LSC) permit low-level radioactivity analysis such as that required in  $^{14}\text{C}$  dating of soil organic matter and soil water flux studies of environmental  $^3\text{H}$ . Advances in microplate scintillation analysis permit the efficient activity analysis of alpha-, beta- and weak gamma-emitting radionuclides with high sample throughput (up to over a thousand samples daily) at reduced cost of consumable materials and waste disposal. Advances in radioisotope activity analysis have been focused on computerized and automated instrumentation with high sample throughput, greater detection efficiencies, refined analysis of radionuclide mixtures, and lower backgrounds with concomitant improved lower limits of detection.

**1. INTRODUCTION**

Radioisotopes continue to be a vital tool as tracers in research in the chemical, biological and agricultural sciences. The writer counted the numbers of research papers in recent issues of the Journal of Biological Chemistry that reported the use of radioisotopes as tracers. Out of 106 research papers in the most recent available journal issue (Vol. 275, No. 21, 2000) 58% reported the use of such tracers, most commonly  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{32}\text{P}$ ,  $^{33}\text{P}$ ,  $^{35}\text{S}$ ,  $^{55}\text{Fe}$ , and  $^{125}\text{I}$ . The basic requirement of this research technique is the determination of the disintegration rate of the radionuclide(s), that is, its absolute activity in curie units (disintegrations per minute, DPM) or Becquerel units (disintegrations per second, DPS). This paper describes state-of-the-art instrumentation and techniques that facilitate the accurate activity analysis of radionuclides commonly used as single or multiple isotope tracers in the agricultural and biological sciences. Attention is also given to advances in the measurement of very low levels of isotope activity such as those encountered in  $^{14}\text{C}$ -dating studies and measurements of environmental  $^3\text{H}$ .

**2. LIQUID SCINTILLATION ANALYSIS VIA A QUENCH INDICATING PARAMETER**

Quench correction in liquid scintillation analysis (LSA) is currently the most common method employed for the activity analysis of radioisotopes that emit beta-particle radiation (e.g.  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{33}\text{P}$ ,  $^{32}\text{P}$ ,  $^{35}\text{S}$ ,  $^{36}\text{Cl}$ , and  $^{45}\text{Ca}$ ) as well as alpha-emitters. It is also used for the analysis of atomic-electron emitting radionuclides, such as  $^{51}\text{Cr}$ ,  $^{55}\text{Fe}$ ,  $^{125}\text{I}$ , and others, which decay via electron capture (EC). The liquid scintillation method currently popular is based upon the measurement of quench effects of sample chemical and colour agents on liquid scintillation pulse height spectra. The sample pulse height spectrum is attenuated in direct proportion to the concentration and strength of sample quenching agent(s) in the liquid scintillation cocktail. An example, illustrated in Fig. 1, demonstrates the effects of increasing amounts of 0.5 M  $\text{HNO}_3$  chemical quenching agent on  $^3\text{H}$  liquid scintillation pulse height spectra and  $^3\text{H}$  detection efficiency in 20 mL of scintillation fluor cocktail.



HNO <sub>3</sub> (0.5M) (mL)	<sup>3</sup> H (CPM)	<sup>3</sup> H (DPM)	<sup>3</sup> H (%E)
0.00	126,287	210,000	60.1
0.05	115,834	210,000	55.1
0.10	102,218	210,000	48.7
0.30	61,211	210,000	29.1
0.50	39,846	210,000	19.0
0.70	25,239	210,000	12.0
1.00	16,091	210,000	7.7

FIG. 1. Pulse height spectra of seven samples of  $^3\text{H}$  of equal activity containing various amounts of 0.5 M  $\text{HNO}_3$  quenching agent. The pulse height spectra are plotted on a logarithmic scale with pulse height calibrated to equivalence keV energy. The liquid scintillation counting (detection) efficiencies for each sample are listed as percentages.

Modern liquid scintillation analysers are programmed to establish a relationship between liquid scintillation detection efficiency for a given radionuclide and quench in the sample by the use of quenched radionuclide standards. The standards used are traceable to a national bureau of standards such as those provided by the National Institute of Standards and Technology (NIST) at Gaithersburg, MD, USA. Because of the relatively long half-lives of  $^3\text{H}$  and  $^{14}\text{C}$ , quenched standards, containing known activities of NIST-traceable  $^3\text{H}$  or  $^{14}\text{C}$  mixed with varying amounts of quenching agent in the scintillation cocktail and contained in suitable counting vials, are available commercially from companies that manufacture liquid scintillation analysers. Quenched standards for other shorter-lived nuclides are available from these suppliers upon request, or they are prepared by the research scientist in the laboratory with a standard of the radionuclide of interest. When assayed in the liquid scintillation analyser, the quenched standards of a given radionuclide provide the relationship of counting efficiency (or detection efficiency) versus a quench indicating parameter (QIP). The QIP is measured by the instrument to provide a mathematical value, which will describe the magnitude and/or shape of the pulse height spectra produced by each of the quenched standards. As illustrated in Fig. 1, the magnitude and shape of the pulse height spectra for seven  $^3\text{H}$  quenched standards show the shifting from higher to lower magnitudes as quench increases. Once the relationship of detection efficiency versus QIP is established for quenched standards, the detection efficiencies of experimental samples of unknown activity are determined from their QIP values.

Liquid scintillation analysers generally are equipped with an external gamma-ray source (external standard) that is automatically placed in close proximity to the liquid scintillation counting vial. The gamma-ray source produces Compton electrons that, in turn, create a pulse height spectrum of magnitude and shape dependent on the quench level in the fluor cocktail. A QIP based on the magnitude and/or shape of the external standard pulse height spectrum is measured. The QIP is a unitless number that describes a certain parameter of the external standard pulse height spectrum such as spectral index, Compton edge, and inflection point. The external standard gamma-ray source and

the QIP measured from its pulse height spectrum differ according to the liquid scintillation analyser manufacturer. Liquid scintillation manufacturers utilize distinct external standard gamma-ray sources and measure different QIPs. Some examples are as follows: (i) Packard Instrument Company using a  $^{133}\text{Ba}$  external standard measuring the transformed spectral index of the external standard referred to as the tSIE, (ii) Beckman Instruments Inc. using a  $^{137}\text{Cs}$ ( $^{137\text{m}}\text{Ba}$ ) external standard measuring the Compton edge of the external standard referred to as the H#, and (iii) EG&G/Wallac using a  $^{152}\text{Eu}$  source measuring the spectral quench parameter of the external standard otherwise known as the SQP(E). Modern liquid scintillation analysers are equipped with computer programs that automate the counting of a series of NIST-traceable quenched standards of a given radionuclide and measurement of the external standard QIP for each quenched standard to establish the relationship of counting efficiency versus the external-standard QIP. This relationship is automatically plotted by the liquid scintillation analyser to be used for automatic quench correction of experimental samples of unknown activity. Figure 2 provides examples of  $^3\text{H}$  and  $^{14}\text{C}$  quench-correction curves made with sets of twenty vials of  $^3\text{H}$  and  $^{14}\text{C}$  quenched standards, respectively.

State-of-the-art liquid scintillation analysers are computer controlled. Quench-correction curves such as those illustrated in Fig. 2 are stored on computer hard disks in appropriate user libraries. Furthermore, the pulse height spectra of all the quenched NIST-traceable standards that are counted are also stored on disk. Therefore, it is usually unnecessary to recount quench standards. This is particularly important whenever a quench-correction curve is needed for a radionuclide of relatively short half-life (e.g.  $^{33}\text{P}$ ,  $^{32}\text{P}$ ,  $^{35}\text{S}$ ,  $^{45}\text{Ca}$ ,  $^{63}\text{Ni}$ ,  $^{89}\text{Sr}$ , and  $^{125}\text{I}$ ). Once the quenched standards are prepared and counted, the pulse height spectrum of each quenched standard is stored automatically on the computer hard disk. Even if changes are made to the counting region (window) such as lower level (LL) and upper level (UL) discriminator settings, the computer will regenerate automatically the new quench-correction curve for the new counting region using the stored pulse height spectra.

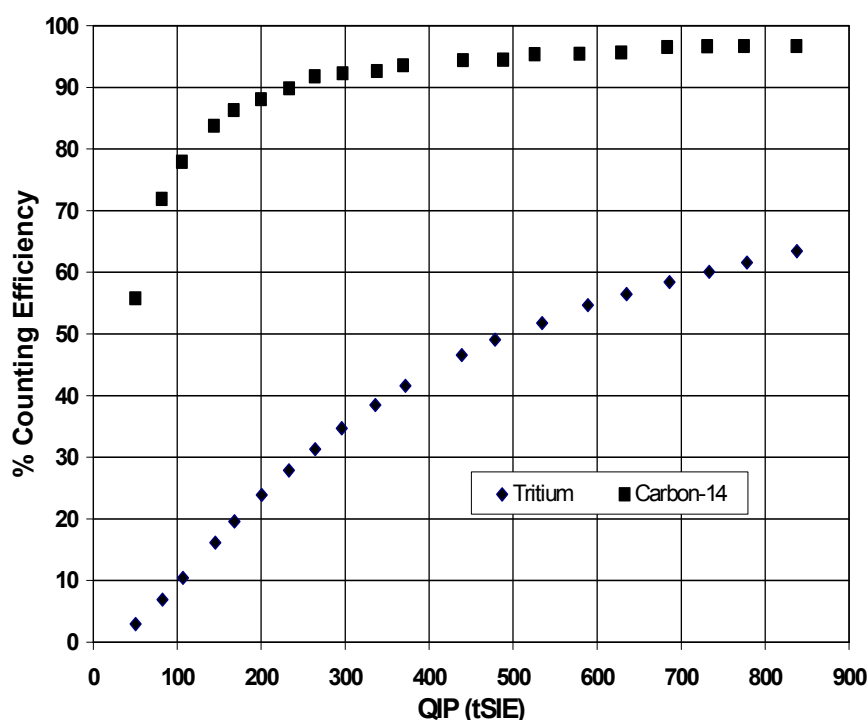


FIG. 2. Quench correction curves for  $^3\text{H}$  and  $^{14}\text{C}$  of detection efficiency versus the external standard quench indicating parameter tSIE plotted automatically by a Packard Tri-Carb 2770 TR/SL liquid scintillation analyser. The  $^3\text{H}$  and  $^{14}\text{C}$  NIST-traceable quenched standards were prepared in Packard Ultima Gold scintillation cocktail and counted in a region of 0–18.6 keV for  $^3\text{H}$  and 0–156 keV for  $^{14}\text{C}$ .

When samples of unknown activity are counted, the modern liquid scintillation analyser will measure the sample count rate (counts per minute or CPM) and also the external standard quench indicating parameter (QIP). From the specific value of the QIP, the computer program extracts the % counting efficiency from the appropriate quench-correction curve similar to one of the curves illustrated in Fig. 2. The absolute activity of the sample is then calculated according to the simple equation

$$DPM_s = \frac{CPM_s}{E}$$

where

DPM<sub>s</sub> is the sample activity in disintegrations per minute (DPM),

CPM<sub>s</sub> is the count rate of the sample (CPM),

and E is the counting efficiency obtained from the quench correction curve expressed as a decimal (e.g. 93% = 0.93).

Once a counting protocol is set up and the quenched standards of an appropriate radioisotope counted, modern liquid scintillation analysers automatically count experimental samples, determine the external standard QIP, sample counting efficiency, and calculate the corresponding sample activity for hundreds of samples without any need for operator attention. Because the QIP is measured via an external gamma-ray source, even low-activity samples (where background count rate is significant) are analysed in a fashion similar to that described above. This method will remain one of the most popular techniques for automatic activity analysis because of its broad versatility for measuring low and high levels of radioactivity. The accuracy of the method can be as good as that of the NIST-traceable standards used to prepare the quench correction curves. This quench-correction method is generally not applied to the activity analysis of alpha-particle emitting radionuclides, because the liquid scintillation analysis detection efficiencies for alpha-particle emissions are near 100% under normal counting modes [1]. More detailed information and procedures on this subject are available in a recent text [1].

### 3. DIRECT DPM METHODS

Although the previously described quench-correction liquid scintillation technique is the most common method employed currently for radioactivity analysis, new direct DPM methods in liquid scintillation analysis have been developed. The direct DPM methods do not require corrections for chemical or colour quench, and have particular applications in the agricultural and biological sciences. Computer driven protocols on certain modern liquid scintillation analysers (e.g. Packard Tri-Carb liquid scintillation counters) are now equipped to provide some automated direct DPM methods of analysis [2]. Two modern direct DPM methods will be described in this section.

#### 3.1. Efficiency tracing DPM

Efficiency tracing DPM (ET-DPM) is a practical and simple extrapolation LSA method for measuring the absolute activity of beta-emitting radionuclides with the exception of tritium. The method was demonstrated by Takiue and Ishikawa [3] to provide accurate DPM values for fourteen radionuclides. A subsequent study by Ishikawa et al. [4] showed that the technique provided accurate DPM measurements of eleven additional beta- and beta-gamma-emitting radionuclides, namely, <sup>14</sup>C, <sup>32</sup>P, <sup>36</sup>Cl, <sup>46</sup>Sc, <sup>59</sup>Fe, <sup>60</sup>Co, <sup>63</sup>Ni, <sup>86</sup>Rb, <sup>90</sup>Sr(<sup>90</sup>Y), <sup>131</sup>I, <sup>134</sup>Cs, and <sup>147</sup>Pm, regardless of quench level.

The method is outlined briefly as follows. Firstly an NIST-traceable <sup>14</sup>C unquenched standard is counted and the % counting efficiencies (% E) in six separate counting regions are recorded. Each region is of different window width starting with the widest counting region possible (e.g. 0–2,000 keV) and subsequent counting regions of narrowing width from the low end of the pulse height spectra (e.g. 2–2,000, 4–2,000, 6–2,000, 8–2,000, and 10–2,000 keV). Subsequently the count rates (CPM) of a sample of a beta-emitting radionuclide of unknown activity are recorded in the same six regions as the NIST-traceable unquenched <sup>14</sup>C standard. The six CPM values of the beta-emitting radionuclide of unknown activity are plotted against the six % E values of the NIST-traceable unquenched <sup>14</sup>C

standard. The plot (curve) is then extrapolated to 100% counting efficiency (100% E), where the CPM of the unknown sample is equal to its DPM. An example of this technique applied to three samples of  $^{35}\text{S}$  of equal activities at three different quench levels is illustrated in Fig. 3. Each curve was made by plotting the CPM of the  $^{35}\text{S}$  samples in six counting regions against the % E of the NIST-traceable unquenched  $^{14}\text{C}$  standard in the same counting regions. The plots were then extrapolated to 100% E. The DPM value of a sample of unknown activity is determined from the linear or multilinear regression equation (best fit) extrapolation to 100% counting efficiency. Recoveries are quantitative, and more detailed information on this technique is available in a recent text [1]. The ET-DPM method is included as an automated analytical direct DPM method on liquid scintillation analysers of the Packard Instrument Company. The method can be applied to the analysis of the disintegration rate of the following type of samples: (a) all beta-emitting radionuclides except tritium, and gamma emissions will not interfere. (b) mixtures of beta-emitting radionuclides where the total DPM of the mixture will be obtained provided tritium is not included in the mixture [5–7], and (c) mixtures of alpha- and beta-emitting radionuclides, again excluding tritium [6]. This efficiency tracing direct-DPM method has some advantages and unique applications: (a) the activities of short-lived radionuclides may be determined for which quench correction curves may not be available, (b) the method may be used to check and/or confirm the radioactivities (DPM) of radioisotope sources that are obtained commercially, prior to the initiation of a radiotracer experiment, (c) the determination of the total DPM of radionuclide mixtures is possible, and (d) the method can be used in combination with Cherenkov counting to determine the separate activities of mixtures of two or even three different radioisotopes [5–7].

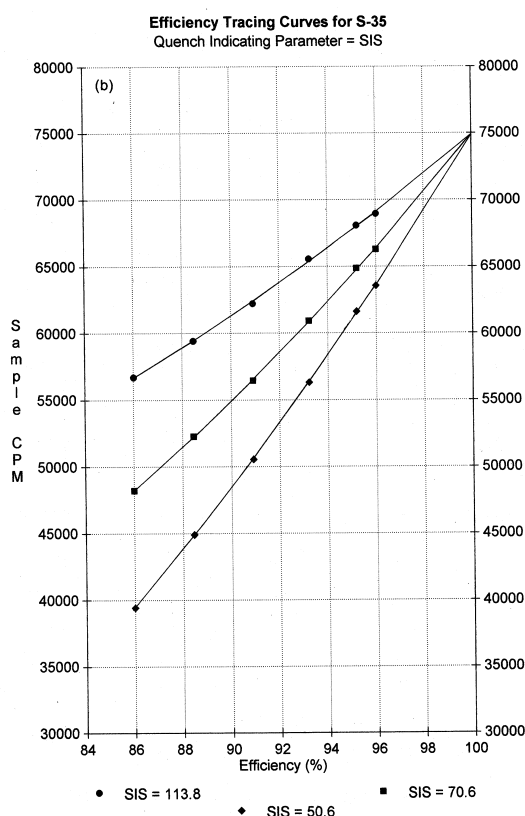


FIG. 3. Efficiency tracing DPM (ET-DPM) plots for three samples of  $^{35}\text{S}$  of the same activity (75,090 DPM) at low, medium and high levels of quench. The levels of quench are indicated by the sample spectrum quench indicating parameters, namely, the spectral index of the sample (SIS). The data were collected with a Packard Tri-Carb 1000 liquid scintillation analyser. (From L'Annunziata et al. [7], reprinted with permission from Elsevier Science.).



The only disadvantage of this technique is that sample count rates must be high, i.e. approximately 100 times background or greater, to provide well defined efficiency tracing curves [2, 7]. This simple method can be used to determine the activity of a short-lived radionuclide. Once the activity of the short-lived radionuclide is determined, a set of quenched standards of that nuclide could then be prepared. With the set of quenched standards a quench correction-curve based on an external standard QIP can easily be produced. With such a quench curve, the disintegration rates of samples of low activity of the short-lived radionuclide can easily be measured.

A practical example of the application of the ET-DPM method can be taken from the work of L'Annunziata and co-workers [7]. The method was applied to the analysis of a mixture of the three radionuclides  $^{86}\text{Rb}$ - $^{35}\text{S}$ - $^{33}\text{P}$ , which may be used as tracers for the elements K-S-P in studies of soil-plant relationships. The close chemical properties of Rb and K permit the use of  $^{86}\text{Rb}$  in many cases as an alternative tracer for K in light of the low commercial availability of  $^{40}\text{K}$  [8]. The ET-DPM method was used firstly to determine the total activity (DPM) of the  $^{86}\text{Rb}$ - $^{35}\text{S}$ - $^{33}\text{P}$  mixture. The  $^{86}\text{Rb}$  activity of the triple-radionuclide mixture was determined by Cherenkov counting in water. Quantitative recoveries for several activity proportions of the triple-radionuclide mixture are illustrated in Table I. The difference in the two measurements provided the activities of the  $^{35}\text{S}$ + $^{33}\text{P}$  mixtures. The activities of the  $^{35}\text{S}$  and  $^{33}\text{P}$  are determined by liquid scintillation analysis of the dual label after the  $^{86}\text{Rb}$  decays to a negligible level according to its half-life (18.8 days) or awaiting for the decay of  $^{33}\text{P}$  (25.3-day half-life). The technique could be used for research applications in crop fertilizer-use efficiency for the major plant nutrients K-S-P. The remaining major nutrient, N, could be studied simultaneously using the stable isotope  $^{15}\text{N}$  as the tracer. The stable isotope, which is analysed by emission or mass spectrometry, would cause no interference in the analysis of the three radionuclides. Consequently, the quadruple isotope label  $^{15}\text{N}$ - $^{86}\text{Rb}$ - $^{35}\text{S}$ - $^{33}\text{P}$  could be utilized as simultaneous tracers for the evaluation of all of the major nutrients of chemical fertilizers. Alternatively, the triple radionuclide mixture may be analysed by one of the liquid scintillation methods described in Section 7.1.

### 3.2. Modified integral counting method

The modified integral counting method (MICM), another state-of-the-art direct DPM method, was reported recently by Homma and co-workers [9, 10], who extrapolated integral counting curves to the zero detection threshold of the liquid scintillation spectrometer, which refers to the average energy required to produce a measurable pulse. They applied the new method to the analysis of alpha- and beta-emitters including  $^{222}\text{Rn}$  and its daughters as well as the low-energy beta emitters  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{35}\text{S}$ , and  $^{45}\text{Ca}$  with 100% detection efficiency.

TABLE I. PERCENT RECOVERIES OF CALCULATED ACTIVITIES OF FIVE COMPOSITE MIXTURES OF  $^{86}\text{Rb}$ - $^{35}\text{S}$ - $^{33}\text{P}$  DETERMINED BY EFFICIENCY TRACING DPM (ET-DPM) TECHNIQUE AND THE COMPONENT ACTIVITIES OF  $^{86}\text{Rb}$  DETERMINED BY CHERENKOV COUNTING AND COMBINED  $^{35}\text{S}$ + $^{33}\text{P}$  OBTAINED BY DIFFERENCE (L'Annunziata et al. [7], reprinted with permission from Elsevier Science)

Sample DPM <sup>a</sup> $^{86}\text{Rb}$ - $^{35}\text{S}$ - $^{33}\text{P}$	Total DPM (actual)	Total DPM (ET)	Total DPM recov. (%)	$^{86}\text{Rb}$ DPM (Cherenkov)	$^{86}\text{Rb}$ recov. (%)	$^{35}\text{S}$ - $^{33}\text{P}$ DPM (difference)	$^{35}\text{S}$ + $^{33}\text{P}$ DPM recov. (%)
4,326:7,294:7,194	18,814	18,671	99.2	4,387	101.4	14,284	98.6
2,146:3,620:3,424	9,190	9,185	99.9	2,199	101.2	6,986	99.2
1,042:1,794:1,550	4,386	4,408	100.5	1,070	102.7	3,338	99.8
3,113:5,510:4,620	13,243	13,237	100.0	3,171	101.9	10,666	99.4
432:743:646	1,821	1,819	99.9	445.5	101.3	1,373	98.9

<sup>a</sup>In hundreds.

Firstly, the method requires the determination of the zero detection threshold of the particular liquid scintillation analyser utilized for the analysis [11]. The integral count rates of an NIST-traceable unquenched  $^3\text{H}$  standard are plotted at several pulse heights, and the curve is extrapolated to the count rate, which is equivalent to the disintegration rate (DPM) of the  $^3\text{H}$  standard. The keV value (pulse height) at this count rate represents the zero detection threshold. The zero detection threshold was found by Homma and co-workers [12] to vary from instrument to instrument over the range of  $2.4$  to  $3.5 \pm 0.2$  keV. Once the zero detection threshold is determined for a particular instrument, the absolute disintegration rate of any beta-emitting radionuclide can be determined by extrapolating the integral pulse height spectrum of the radionuclide sample of interest to the previously determined zero detection threshold. Examples of results obtained with the modified integral counting method applied to the activity determination of  $^{35}\text{S}$  and  $^{45}\text{Ca}$  are illustrated in Fig. 4.

The MICM was reported also by Homma and co-workers [13, 14] for the determination of  $^{222}\text{Rn}$  and its daughters  $^{218}\text{Po}$ ,  $^{214}\text{Pb}$ ,  $^{214}\text{Bi}$ , and  $^{214}\text{Po}$ . Total alpha- and beta activity are determined with 100% counting efficiency. The MICM can be applied to the activity measurements of alpha- and beta emitters as single radionuclide samples or mixtures for total DPM, and gamma emission does not interfere in most cases [12]. However, the method is not used for low-level counting (i.e. count rates near background up to  $\sim 2 \times 10^3$  CPM). For low-level counting and for the analysis of samples of higher activity, the liquid scintillation analysis method via a quench indicating parameter (QIP, Section 2) will remain the mainstay for many years to come.

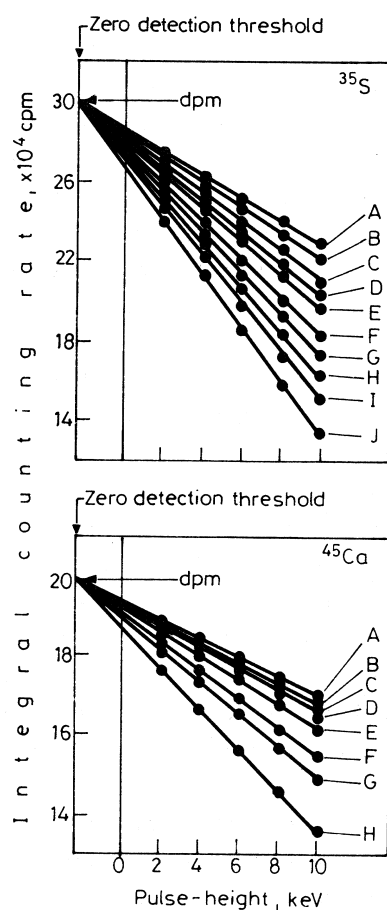


FIG. 4. Extrapolation plots of the integral count rates of quenched  $^{35}\text{S}$  and  $^{45}\text{Ca}$  to the zero detection threshold for determination of the radionuclide disintegration rates. Letters A, B, C, ... denote samples with increasing quench levels in Packard Ultima Gold liquid scintillation cocktail. Deviations from actual DPM values were  $< 1\%$  for all plots. (From Homma et al. [12], reprinted with permission from Elsevier Science.).

#### 4. LOW-LEVEL LIQUID SCINTILLATION ANALYSIS VIA TIME-RESOLVED LIQUID SCINTILLATION COUNTING

During the early years of  $^{14}\text{C}$ -dating, researchers in low-level counting had to resort to radiation detectors surrounded with extensive lead shielding, counting laboratories located in underground or basement facilities, clumsy anti-coincidence circuitry linked with gas-ionization detectors, and tedious manual operation for radiation counting and sample changing. Today, very-low-background count rates required for  $^{14}\text{C}$  dating and environmental  $^3\text{H}$  measurements are achievable with some modern liquid scintillation analysers that can obviate all of the above disadvantageous working conditions including the need for no additional lead shielding than that used for any conventional liquid scintillation analyser. The recent advances made in this field are covered in this section.

The greatest advancement made in the field of low-level counting in the past decade is time-resolved liquid scintillation counting (TR-LSC) in conjunction with the use of a bismuth germanate (BGO) scintillation detector guard. TR-LSC is a patented method (Packard Instrument Co.) for reducing background by discriminating against pulses on the basis of the number of afterpulses that occur following an initial pulse event over a given period of time. Afterpulses are generally more numerous among non-quenchable background events and less numerous with beta-particle events that occur in the scintillation cocktail (quenchable events). The sharp contrast between the number of afterpulse events that occurs as a consequence of typical beta-particle scintillation in the cocktail versus the afterpulse events that occur as a consequence of background is illustrated in Fig. 5. A pulse event resulting from a typical quenchable beta-particle interaction in scintillation cocktail has few, if any, afterpulses; whereas, a typical non-quenchable background event will produce many afterpulses [15–18].

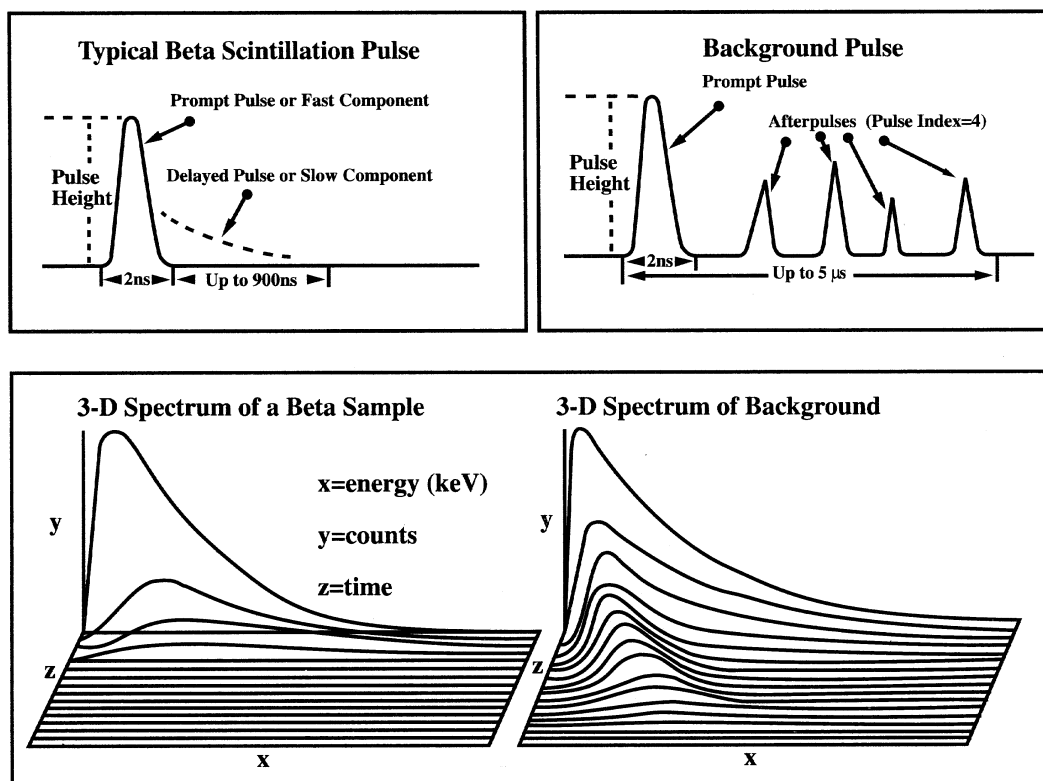


FIG. 5. Comparison of typical beta-particle induced quenchable scintillation pulse and non-quenchable background pulse in two dimensions (upper left and right graphs, respectively) and three dimensions (lower diagrams). The pulse index discrimination can be set to count a specific number of afterpulses to discriminate between beta and background events. (Courtesy of the Packard Instrument Co., Meriden, CT, USA.).

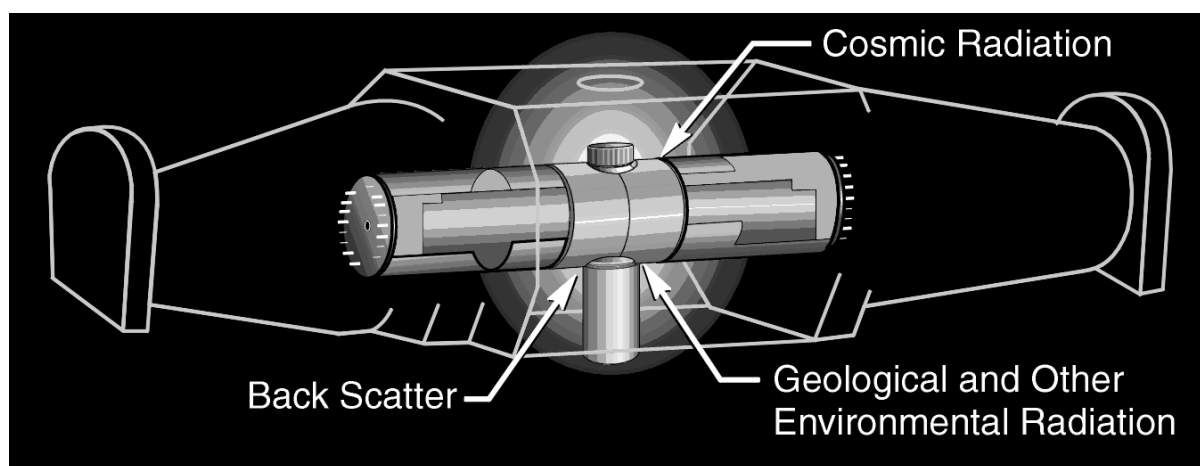


FIG. 6. Schematic representation of the BGO detector guard, passive shield, and detector chamber in the Packard 2770TR/SL and 3170TR/SL liquid scintillation analysers [16].

With the TR-LSC technique, the liquid scintillation analyser is manufactured and programmed to count afterpulses, referred to as pulse index discrimination, which can identify background events with varying degrees of sensitivity. Among various Packard Tri-Carb liquid scintillation analysers, the degree of pulse index discrimination for background rejection can be factory set or programmed to provide different sensitivities for the reduction of background count rates. Examples of degrees of sensitivity of pulse index discrimination are (a) normal TR-LSC, (b) high-sensitivity TR-LSC, (c) low-level TR-LSC, and (d) ultra low-level TR-LSC with BGO detector guard. The latter and most sensitive level of background discrimination involves a liquid scintillation analyser that is equipped with a BGO solid scintillator detector guard mounted in the counting chamber that surrounds the sample liquid scintillation counting vial. The BGO guard facilitates the detection and rejection of non-quenchable pulse events by enhancing further the intensity and number of afterpulses produced by external background radiation such as cosmic radiation, back-scatter radiation, and geological and other environmental radiation not originating from the sample scintillation cocktail. Figure 6 illustrates the location of the BGO detector guard relative to the sample vial and photomultiplier tubes.

Tables II and III illustrate how significantly background can be reduced and performance enhanced using TR-LSC. The data in these tables can be found in a typical low-level counting laboratory. The data of Table II illustrate that the use of TR-LSC alone, and in combination with a BGO active detector guard, increases the performance of the liquid scintillation analyser. For the measurement of environmental  $^{14}\text{C}$ , such as in  $^{14}\text{C}$ -dating, a 15- to 20-fold performance enhancement is obtained with the ultra-low-level TR-LSC and the BGO detector guard.

TABLE II. RADIOCARBON DATING SAMPLE AS 3.5 mL BENZENE WITH PPO/POPOP SCINTILLATOR<sup>a</sup> [19]

Amount of pulse index discrimination	Detector guard	$^{14}\text{C}$ efficiency (%)	Background (CPM)	Figure of merit ( $\text{E}^2/\text{B}$ )
None	No	83	9.67	720
Normal TR-LSC	No	82	7.07	948
High-sensitivity TR-LSC	No	79	4.74	1,300
Low-level TR-LSC	No	70	1.38	3,560
Ultra-low TR-LSC+BGO	Yes	65	0.33	12,675

<sup>a</sup>The optimized counting region is 10–102 keV with various levels of TR-LSC pulse index discrimination applied to the pulse events. (Courtesy of the Packard Instrument Company, Meriden, CT.)

TABLE III. ENVIRONMENTAL TRITIUM WATER SAMPLE AS 10 mL WATER IN 10 mL ULTIMA GOLD LLT SCINTILLATION COCKTAIL<sup>a</sup> [19]

Amount of pulse index Discrimination	Detector guard	<sup>3</sup> H efficiency (%)	Background (CPM)	Figure of merit (E <sup>2</sup> /B)
None	No	26	18.4	38
Normal TR-LSC	No	26	12.7	54
High-sensitivity TR-LSC	No	25	9.25	66
Low-level TR-LSC	No	23	3.33	153
Ultra-low TR-LSC+BGO	Yes	20	1.00	400

<sup>a</sup>The optimized counting region is 0.5–5.0 keV with various levels of TR-LSC pulse index discrimination applied to the pulse events. (Courtesy of the Packard Instrument Company, Meriden, CT, USA)

The data in Table III illustrate that the use of TR-LSC alone and in combination with a BGO active detector guard greatly increases the performance of the liquid scintillation analyser for the measurement of environmental tritium samples, or tritium samples in general with low count rates. In the case of tritium, about a 10-fold better performance is achieved with ultra low level TR-LSC in combination with the BGO detector guard.

Studies on the mean residence time or age of soil organic matter are carried out with the use of the <sup>14</sup>C-dating technique [20]. Also, studies have been reported on environmental tritium in the estimation of soil water movement and of the age of aquifer water [21]. Measurements of environmental <sup>14</sup>C and <sup>3</sup>H once required tedious procedures to reduce background count rates such as extensive lead shielding, anti-coincidence detectors, and low-background underground counting laboratories. However, a conventional modern liquid scintillation analyser can do the job when equipped with pulse index discrimination and a BGO detector guard for background rejection. The same equipment can be used to analyse higher levels of radioactivity encountered in general tracer studies with isotopes such as <sup>32</sup>P. We should also keep in mind that the reduced background count rates provided by instruments equipped with low-level TR-LSC or ultra-low-level TR-LSC + BGO guard will yield improved lower limits of detection (LLD) for radioisotopes. This would permit longer periods of research with radioisotopes of relatively short half-life, such as <sup>32</sup>P.

## 5. CHERENKOV COUNTING

Phosphorus-32 is commonly used as a tracer in studies of fertilizer-use efficiency, and in molecular biology and biochemistry. Liquid scintillation analysis of <sup>32</sup>P remains a common practice because of the high detection efficiencies (~100%) achieved over a wide range of quench due to high energy of the beta-particle emissions ( $E_{\max} = 1,710$  keV). Nevertheless, evidence from recent work will be provided here to demonstrate that the Cherenkov counting of photons produced by <sup>32</sup>P in water, with a modern liquid scintillation analyser, will provide improved lower limits of detection of <sup>32</sup>P without the cost that is incurred with liquid scintillation cocktail use and disposal.

The optimum sample volumes and efficiencies for the counting of Cherenkov photons produced by <sup>32</sup>P in water will differ for polyethylene plastic and glass counting vials, as illustrated in Fig. 7. Polyethylene plastic vials provide both a higher detection efficiency and a reduced geometry effect of sample volume on detection efficiency. The graphs illustrated in Fig. 7 may vary from one instrument to another, and the exact volume effects for a particular instrument should be determined experimentally. If we compare polyethylene plastic vials with glass in terms of detection efficiency and background we see that the former provide improved performance as measured by the figure-of-merit (FOM) values provided in Table IV.

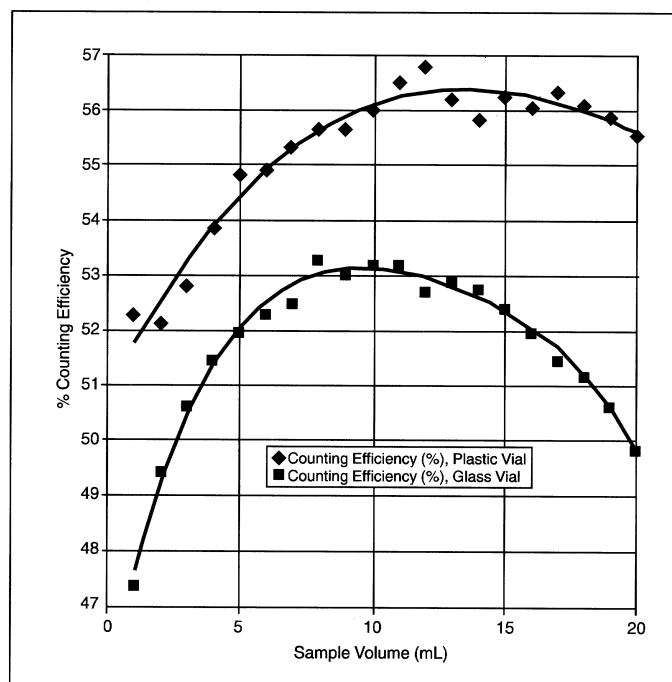


FIG. 7. Effect of sample volume on the Cherenkov counting efficiency of  $^{32}\text{P}$  in water with polyethylene plastic and glass counting vials measured with a Packard Tri-Carb 2300TR liquid scintillation analyser [22, 23].

TABLE IV.  $^{32}\text{P}$  CHERENKOV COUNTING PERFORMANCE [23]

Vial type	Count mode	Counting efficiency (%)	Background <sup>a</sup> (CPM)	Figure of merit ( $E^2/B$ )
Plastic <sup>b</sup>	NCM <sup>c</sup>	56.7	16.6	193.7
Glass	NCM	50.2	29.1	86.6

<sup>a</sup>Background measurements were made with triplicate samples of pure water in the counting region 0–30 keV (LL–UL) and counted for 200 min each with a %2 sigma (%2S) standard deviation of 3.0% with a Packard Tri-Carb 2300TR liquid scintillation analyser. <sup>b</sup>Polyethylene. <sup>c</sup>NCM represents normal count mode or normal TR-LSC.

If we consider that Cherenkov counting does not require scintillation fluor cocktail, a 20-mL sample volume can be counted in a standard 20-mL counting vial. On the contrary, in LSA it is necessary to mix sample with scintillation fluor cocktail, and in such a circumstance the largest sample volume that can be accommodated in a 20-mL vial is 10 mL, i.e. 10 mL sample + 10 mL cocktail. The larger sample volume that can be counted with the Cherenkov counting technique yields an improved minimum detectable activity (MDA) over LSA calculated on the basis of differences in counting efficiency and sample volume, as illustrated in Table V. Other advantages of Cherenkov counting over LSA of  $^{32}\text{P}$  are (a) no additional costs for scintillation fluor cocktail use and disposal, as illustrated in Table IV, (b) no interferences from other low-energy beta-emitting radionuclides that may be in the sample (e.g.  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{33}\text{P}$ ,  $^{35}\text{S}$ ), and (c) samples analysed by Cherenkov counting are not contaminated with fluor cocktail. Because cocktail is not present, further studies may be carried out on these samples, such as total P analysis required in fertilizer use efficiency studies, and/or other chemical or spectrometric analysis required in the biological sciences. In summary, there is no reason or argument in favour of LSA over Cherenkov counting for the analysis of  $^{32}\text{P}$  activity.

TABLE V. COMPARISON OF LIQUID SCINTILLATION ANALYSIS (LSA) AND CHERENKOV COUNTING OF  $^{32}\text{P}$

Method	Counting efficiency (% E)	Largest sample volume (mL)	MDA <sup>a</sup> (pCi/L)	Cocktail use expense	Cocktail disposal expense
LSA	100	10	26.9	Yes	Yes
Cherenkov	57 <sup>b</sup>	20	23.6	No	No

<sup>a</sup>Minimal Detectable Activity or Lower Limit of Detection calculated according to the values of % E and sample volume provided above, 100-min sample and background counting time, and a background of 16.6 CPM from Table III [15, 19, 24]. <sup>b</sup>Cherenkov counting efficiency of  $^{32}\text{P}$  in water and polyethylene plastic counting vial (Table III).

## 6. MICROPLATE SCINTILLATION ANALYSIS

Research studies with radioisotopes can require high-throughput, entailing the analysis of over a thousand samples per day. Examples are radioisotope applications in large-scale fertilizer-use-efficiency studies, the screening of compounds or reactions in molecular biology such as in the search for new therapeutic agents and drug candidates, and the assessment of molecular biology functions. If traditional LSA were used for these studies, the numerous samples involved would require large numbers of vials, and large quantities of fluor cocktail and radioactive waste. This adds considerable cost to sample analysis and disposal. Consequently, microplate scintillation counting instruments, in combination with automatic liquid-handling devices, have been developed to increase sample throughput via the automated simultaneous analysis of several samples with multiple detectors, and to reduce cost of consumable materials and waste disposal [19, 25].

The Packard TopCount microplate scintillation and luminescence analyser uses a microplate sample format designed to permit the analysis of large numbers of samples in a relatively small format. The microplate is a small container with many sample wells. About the size of a postcard, 12.7×8.6×1.9 cm (L×W×H), the microplate comes in three sample-well formats, namely a 24-well plates (6×4 wells) illustrated in Fig. 8, an 96-well plate (12×8), and a 384-well plate (24×16). The 24-well microplate holds up to 375 and 1,500  $\mu\text{L}$  per well for shallow- and deep-well designs, respectively. The 96-well microplate holds 75 and 350  $\mu\text{L}$  per well for shallow- and deep-well designs, respectively, and the 384-well microplate holds up to 80  $\mu\text{L}$  per well. It is clear that only relatively small samples can be analysed in these microplates, but the footprint is small, compact, and easy to automate. As a result of small sample sizes and small amounts of scintillation cocktail required, the activity analysis of many samples can be carried out without consuming large quantities of cocktail or producing large amounts of radioactive waste. The microplate scintillation counter can count up to twelve samples simultaneously directly in the microplate. The stacking of forty microplates containing 384 sample wells per plate permits the unattended analysis of up to 15,360 samples.

The most efficient detector arrangement for the microplate scintillation and luminescence counter is that which utilizes one photomultiplier tube (PMT) per sample, which, with twelve PMTs, is capable of analysing twelve samples simultaneously, as employed in the Packard TopCount microplate scintillation and luminescence counter. A white, reflective, opaque microplate is utilized whereby light produced by the radioactivity in the liquid scintillator is reflected back toward the PMT and no optical cross-talk occurs, as illustrated in Fig. 9.

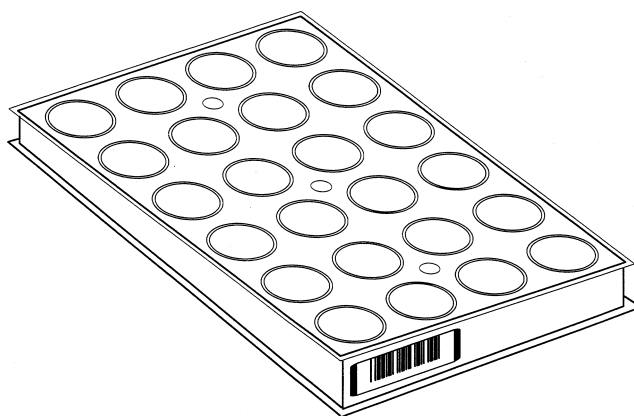


FIG. 8. A 24-well microplate (6×4) measuring approximately 12.7×8.6×1.9 cm. A bar-code label on one side of the plate is used to provide identification of each plate within a computer-controlled assay. The TopCount microplate scintillation and luminescence analyser reads the label and assigns a number to the plate to provide a positive identification of sample numbers with sample radioactivity results. (Courtesy of Packard Instrument Co., Meriden, CT 06450, USA).

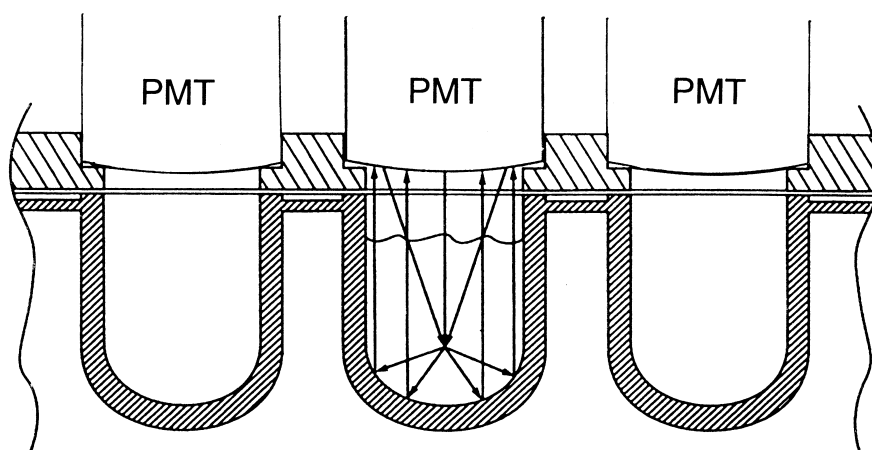


FIG. 9. Cross section of a microplate segment illustrating three white opaque wells and photomultiplier tubes (PMTs) aligned above each, utilizing only one PMT per well and up to twelve PMTs to analyse simultaneously as many as twelve samples. (Courtesy of the Packard Instrument Co., Meriden, CT 06450, USA.)

Where a single PMT is employed to detect scintillation photons above a well, modern advances made in time-resolved liquid scintillation counting (TR-LSC) provide good background reduction. The single-PMT TR-LSC technique, employed in the Packard TopCount microplate scintillation counter, uses pulse counting to distinguish between true scintillation pulse events and background noise. This obviates the need for a second PMT per sample and reduces lead-shielding requirements. The elimination of a second PMT per sample also facilitates close physical alignment of PMTs for the simultaneous counting of up to twelve samples on a single microplate. Single-PMT counting with TR-LSC uses scintillators of relatively long decay constant. Such scintillators emit photons, after beta-particle excitation, over a longer period of time. Each scintillation pulse produces a photon packet followed by a series of pulses, as illustrated in Fig. 10, whereas PMT noise creates single-pulse events. In modern TopCount TR-LSC, the characteristics of a pulse are determined over a period of time (e.g. 200 ns) after the initial packet is detected. If it is followed by one or more additional pulses within the resolving time period of 200 ns, the pulse is accepted as a true scintillation event. If no additional pulses are detected within the resolving time period, the initial pulse is rejected as background noise. The resolving time circuit to initiate pulse decay discrimination is triggered when a pulse height exceeds the single photon event (SPE) threshold. The number of pulses above the SPE threshold is



counted during the resolving time period. Multiple pulses detected in the resolving time are accepted as a valid event, which is further analysed in the pulse height analyser of the liquid scintillation counter. If multiple pulses are not detected in the resolving time interval, the triggering pulse is rejected as background noise.

This method of rejecting background noise pulses provides acceptable background count rates. Optimum counting efficiencies (% E) and background count rates (CPM) reported for some radionuclides commonly used in the agricultural and biological sciences are as follows: (a)  $^3\text{H}$ , 50% E, 18 CPM, (b)  $^{14}\text{C}$ , 92% E, 18 CPM, (c)  $^{32}\text{P}$ , 81% E, 8 CPM, (d)  $^{51}\text{Cr}$ , 21% E, 18 CPM, and (e)  $^{125}\text{I}$ , 58% E, 18 CPM. With the single PMT design described above, where the PMT is located above the microplate sample well, an external gamma-ray standard can be used for automated quench correction and DPM determinations of samples. The external standard can be positioned automatically by the instrument below the sample well of the microplate when needed for automatic quench correction. This method of quench correction is described in Section 2 of this paper. It is particularly applicable as a quench-correction method for samples of low- or high-count rates, and in the measurement of dual radionuclide mixtures.

There is a growing interest in microplate scintillation counting particularly where large-scale research projects with radioisotopes require a high sample throughput of as many as over a thousand sample activities analysed per day by the liquid scintillation technique at reduced chemical and waste disposal costs. These include large-scale fertilizer-use-efficiency studies, pesticide and drug screening, wipe-test applications, and general biological applications, such as cell-proliferation assays, enzyme-inhibition assays and microplate immunoassays [19]. Solid scintillation counting of beta-particle and Auger-electron emitting radionuclides in microplates is popular, as described in Section 8.2.

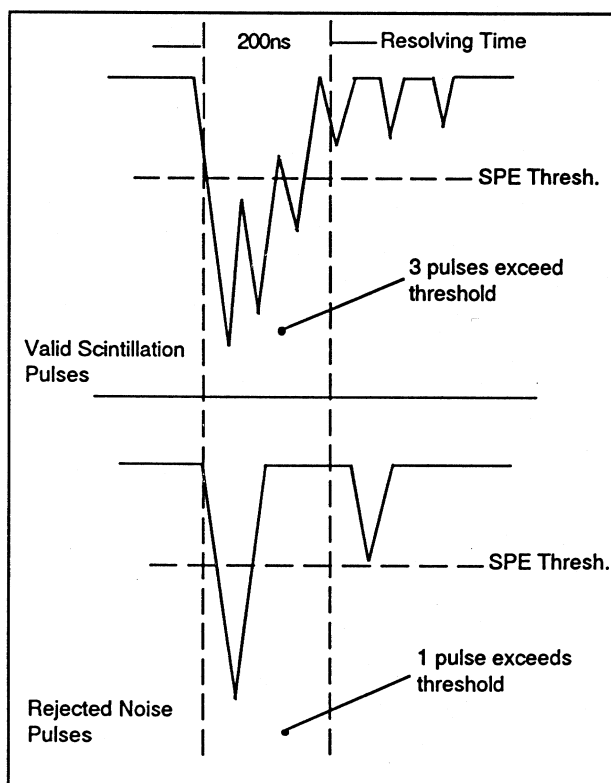


FIG. 10. Single PMT time-resolved pulse discrimination employed in the Packard Top Count Microplate Scintillation and Luminescence Counter.

## 7. MULTIPLE RADIONUCLIDE ANALYSIS

Double label radionuclide tracers are commonly used in the agricultural, biological, and environmental sciences [19]. The dual radionuclide mixtures most frequently used include  $^3\text{H}$ - $^{14}\text{C}$ ,  $^3\text{H}$ - $^{32}\text{P}$ ,  $^3\text{H}$ - $^{35}\text{S}$ ,  $^{14}\text{C}$ - $^{32}\text{P}$ ,  $^3\text{H}$ - $^{125}\text{I}$ ,  $^{33}\text{P}$ - $^{32}\text{P}$ ,  $^{35}\text{S}$ - $^{32}\text{P}$ ,  $^{125}\text{I}$ - $^{131}\text{I}$ ,  $^{51}\text{Cr}$ - $^{14}\text{C}$ ,  $^{67}\text{Ga}$ - $^{68}\text{Ga}$ ,  $^{55}\text{Fe}$ - $^{59}\text{Fe}$ ,  $^{125}\text{I}$ - $^{14}\text{C}$ ,  $^{59}\text{Fe}$ - $^{51}\text{Cr}$ , and  $^{89}\text{Sr}$ - $^{90}\text{Sr}$  [9, 26]. Applications of triple radioisotope labels as tracers in the agricultural and biological sciences are less common, but examples such as  $^3\text{H}$ - $^{14}\text{C}$ - $^{32}\text{P}$  and  $^3\text{H}$ - $^{14}\text{C}$ - $^{36}\text{Cl}$  are found in the literature [19, 26, 27]. More complex mixtures are possible, and modern methods of analysis of these are discussed subsequently.

### 7.1. Conventional dual and triple DPM analysis

Since the early years of liquid scintillation analysis to the current day, dual beta-emitting radionuclide mixtures have been analysed in two counting regions defined by lower level (LL) and upper level (UL) pulse height discriminator settings. In these two counting regions, there is scintillation pulse height spillover of both radionuclides. This old method is often referred to as the conventional dual-label DPM method [1, 19, 28, 29]. It is reliable in terms of radioisotope recovery, but can be tedious in defining the best counting regions, and the % counting efficiencies of the two radionuclides are not at an optimum, because of the isotope spillover. The method is also applied to the analysis of three beta-emitting radionuclides in a mixture, which would require three counting regions. A more modern method that facilitates and optimizes dual radionuclide analysis has been developed, the full-spectrum DPM (FS-DPM) method.

#### 7.1.1. Full-spectrum DPM analysis

The full-spectrum DPM method is a user-friendly patented technique provided with Packard liquid scintillation analysers for the measurement of many dual-label radionuclide combinations such as those listed in the introduction to this section. The method is simple because no counting regions need to be defined to separate the two radionuclides of the mixture. The FS-DPM method provides the deconvolution of the composite pulse height spectra of two radionuclides within a single wide-open pulse height counting region, as illustrated in Fig. 11. The key to the method is the quench-indicating parameter of the sample spectrum, namely the spectral index of the sample, SIS. At a given level of quench, each radionuclide has a defined pulse height-energy distribution and hence a unique SIS value. When two radionuclides are combined into a single sample, the resultant composite pulse height is the sum of the two individual distributions. The SIS of the total distribution ( $\text{SIS}_T$ ) is a function of the SIS of the individual distributions and the fractional counts of each radionuclide as demonstrated by L'Annunziata and co-workers [30, 31]. If  $\text{SIS}_L$  and  $\text{SIS}_H$  are the spectral index values of the low- and high-energy radionuclides of a dual-radionuclide sample, then the SIS of the total distribution  $\text{SIS}_T$  is calculated as:

$$\text{SIS}_T = \frac{(\text{SIS}_L)(\sum N_L E + \text{SIS}_H)(\sum N_H E)}{\sum N_L E + \sum N_H E} \quad (7.1.1)$$

where

$\sum N_L E$  is the accumulated counts from the low-energy radionuclide (e.g.  $^3\text{H}$ ),  
and  $\sum N_H E$  is the accumulated counts from the high-energy radionuclide (e.g.  $^{14}\text{C}$ ).

From Eq. 7.1.1, the count rates of the low-energy radionuclide ( $\text{CPM}_L$ ) and the high-energy radionuclide ( $\text{CPM}_H$ ) of a composite sample are derived. The disintegration rates of the two radionuclides in the mixture are determined automatically from external standard quench-correction curves.

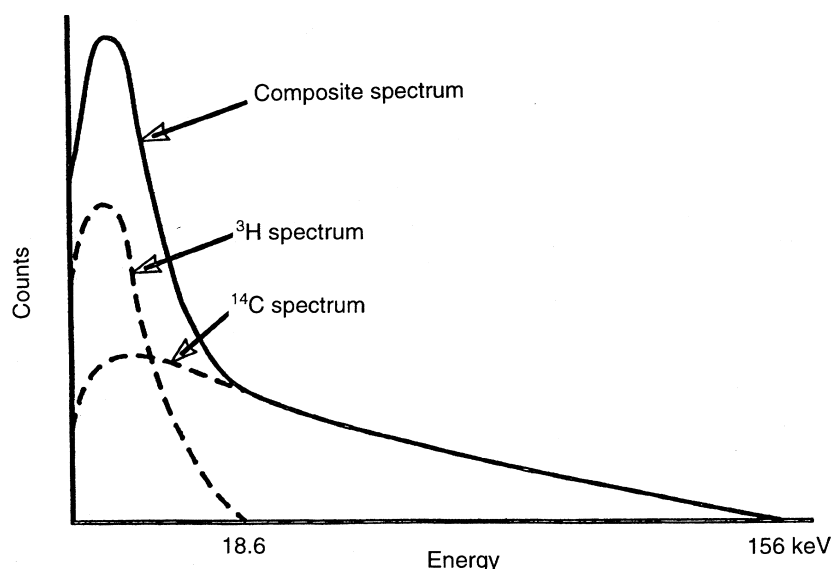
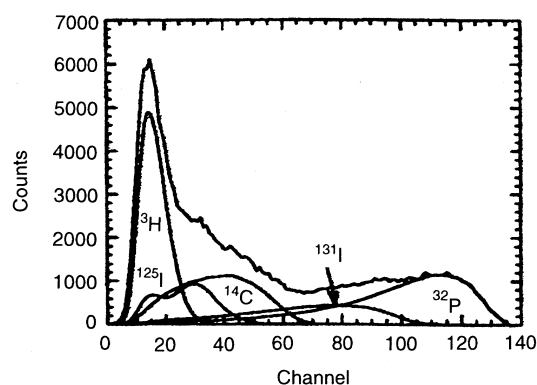


FIG. 11. Composite spectrum of a mixture of  $^3\text{H}$  and  $^{14}\text{C}$  (solid line) defined by the value of  $\text{SIS}_T$  of equation 7.1, and the deconvoluted spectra of  $^3\text{H}$  and  $^{14}\text{C}$  (broken lines) defined by the values of  $\text{SIS}_L$  and  $\text{SIS}_H$ , respectively, of Eq. 7.1.1 [19].

The FS-DPM method for the analysis of dual radionuclide mixtures has the following advantages over the conventional dual DPM method: (a) FS-DPM provides higher counting efficiencies because a full wide-open counting region is used, and (b) it is easy to set up in the liquid scintillation analyser because counting regions need not be defined. The FS-DPM method requires an instrumental measurement of the spectral index of the sample ( $\text{SIS}_T$ ). Therefore, sample counts  $>1,000$  CPM are recommended for accurate measurements. Quantitative recoveries of the activities (DPM) of the dual radionuclides in the mixture within 1% accuracy are reported [29]. For additional information, numerous references can be cited [1, 19, 29, 32–34]. When sample count rates are low ( $<1,000$  CPM), the conventional dual DPM method described in Section 7.1. is used.

### 7.1.2. More-complex radionuclide mixtures

From the beginning of liquid scintillation analysis in the 1950s up to about 1990, the analysis of more than three beta-emitting radionuclides in the same sample was considered to be impracticable or not feasible. However, with technical advances including applications of multichannel analysers in liquid scintillation counters and direct computer processing of LSA data, the analysis of numerous beta- and beta-gamma-emitting radionuclides in a mixture has become feasible. The major advances in this field have been the following (a) the application of the most-probable-value theory to the simultaneous activity analysis of as many as seven radionuclides (i.e.,  $^{51}\text{Cr}$ - $^3\text{H}$ - $^{125}\text{I}$ - $^{14}\text{C}$ - $^{45}\text{Ca}$ - $^{22}\text{Na}$ - $^{32}\text{P}$ ) in a mixture [35–40], (b) the use of spectral deconvolution and interpolation methods for the activity analysis of multiple radionuclides in a mixture, as illustrated in Fig. 12 [41–51], (c) the spectral unfolding of alpha- and beta-emitters in one liquid scintillation multichannel analyser without alpha/beta discrimination [51, 52], and (d) the application of multivariate calibration to the liquid scintillation deconvolution of alpha-emitting radionuclides in the same sample [53–54]. Several radionuclides of interest as tracers in the agricultural and biological sciences can now be analysed as complex mixtures for multi-tracer studies with the use of a standard modern liquid scintillation analyser and personal computer for data processing of pulse height spectra. The mean percent recoveries for mixtures of  $^3\text{H}$ - $^{14}\text{C}$ - $^{45}\text{Ca}$ - $^{32}\text{P}$  at different quench levels with a Packard Tri-Carb 460C liquid scintillation analyser was 2.4% [40], and for the analysis of six radionuclides,  $^3\text{H}$ - $^{63}\text{Ni}$ - $^{14}\text{C}$ - $^{45}\text{Ca}$ - $^{36}\text{Cl}$ - $^{32}\text{P}$ , the mean recovery using a Packard Tri-Carb 4000 liquid scintillation analyser and quench correction using tSIE as the QIP was reported to be 3.9% [37–38], and for low-level analysis of seven radionuclides,  $^{51}\text{Cr}$ - $^3\text{H}$ - $^{125}\text{I}$ - $^{14}\text{C}$ - $^{45}\text{Ca}$ - $^{22}\text{Na}$ - $^{32}\text{P}$ , the lower limit of detection was calculated as  $0.01 \text{ Bq mL}^{-1}$  for higher energy radionuclides and  $0.05 \text{ Bq mL}^{-1}$  for lower energy radionuclides in the mixtures [39].



Local Quench Parameters and Efficiency				
Nuclide	Quench	Maximum	End	Efficiency
P32	280.8	112.0	136.8	0.9964
I131	280.8	76.6	113.4	0.9620
I125	280.8	29.1	54.1	0.6115
C14	280.8	40.5	71.9	0.8647
H3	280.8	13.7	35.5	0.2129

Nuclide	Counting rate (cpm)
P32	27291.9
I131	12298.1
I125	11669.4
C14	21525.8
H3	28965.3

Nuclide	Activity (dpm)	Uncertainty (%)
P32	27391.1	0.5
I131	12784.0	2.3
I125	19083.2	3.4
C14	24893.0	1.9
H3	136049.3	2.2

FIG. 12. Separated components of a composite mixture of  $^3\text{H}$ - $^{14}\text{C}$ - $^{125}\text{I}$ - $^{131}\text{I}$ - $^{32}\text{P}$  and the underlying computer output of activity analysis by liquid scintillation spectrum unfolding. From Grau Carles [44], reprinted with permission from Elsevier Science.

Quantitative recoveries of five radionuclides in a mixture, i.e.  $^3\text{H}$ - $^{125}\text{I}$ - $^{14}\text{C}$ - $^{131}\text{I}$ - $^{32}\text{P}$ , by liquid scintillation spectrum unfolding are illustrated in Fig. 12. [44]. The reader is invited to refer to detailed treatments on the subject [1, 19] and to the literature cited above for more details on the techniques involved.

## 8. SOLID SCINTILLATION ANALYSIS

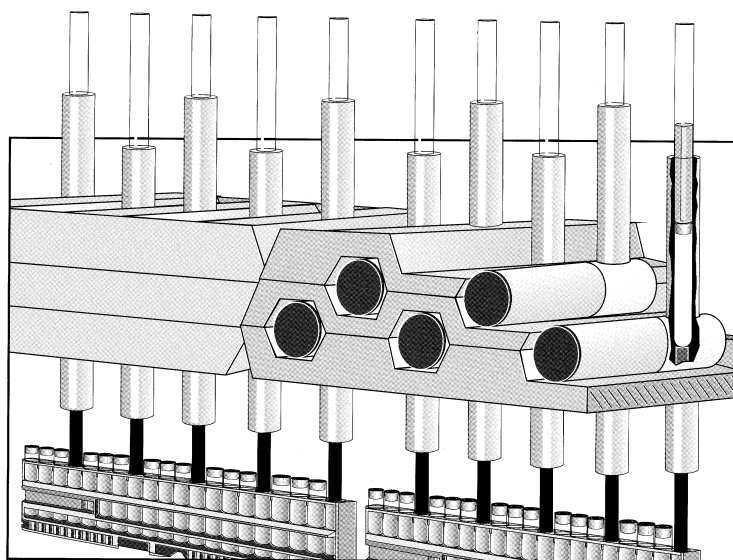
Modern advances in solid scintillation analysis of interest in the agricultural and biological sciences have been in the field of automated analysers referred to as high-throughput computer-based multiple-detector instruments capable of analysing simultaneously ten to twelve samples with automated instrument performance assessment, multiple-user programming, “walk away” sample analysis, and “hands-off” data processing of samples with potential links to computer mainframe and local area network (LAN) assay automation and data management. Two types of automated state-of-the-art solid scintillation analysers are (a) automated gamma analysers that utilize up to 10 NaI(Tl) detectors for the simultaneous measurement of gamma- and x-ray-emitting radionuclides in 750 to 1,000 sample tubes, and (b) microplate scintillation analysers that utilize 24-, 96-, and 384-well microplates for the solid or liquid scintillation analysis of beta particle- and Auger electron-emitting radionuclides with the potential for the unattended analysis of up to 15,360 samples. These two types of solid scintillation

analysers are described below in brief. For more detailed information the reader may consult lengthy reviews in the literature [1, 55].

### 8.1. Automated NaI(Tl) gamma analysers

Modern automated solid scintillation analysers provide for the unattended analysis of large volumes of samples containing gamma- and x-ray-emitting radionuclides. Among the main components are (a) a large-capacity sample changer capable of holding and moving up to 1,000 sample tubes to and from detectors, (b) multiple detectors for the simultaneous activity analysis of up to ten samples (see Fig. 13), (c) automatic computer-controlled system with numerous independent programmable assay protocols, (d) sample-number identification via binary code, which can be linked to a local area network data processing and management system, (e) automatic isotope decay correction, (f) correction for radionuclide interference between counting regions via automatic spillover calculations, (g) multiple simultaneous counting regions preset for radionuclides such as  $^{125}\text{I}$ ,  $^{57}\text{Co}$ ,  $^{60}\text{Co}$ ,  $^{75}\text{Se}$ ,  $^{51}\text{Cr}$ ,  $^{131}\text{I}$ ,  $^{59}\text{Fe}$ , and  $^{22}\text{Na}$  or arbitrarily definable with LL and UL discriminator settings over a wide energy region (e.g., 15–2,000 keV), (h) automatic instrument performance assessment, and (i) applications software for sample data processing and calculations.

Modern solid scintillation analysers like liquid scintillation analysers are computer controlled, that is, all instrument operation and data analysis is via the computer. The user defines analysis protocols, count-condition parameters for the control of sample counting, and data-reduction parameters, which include assay-specific data calculations, curve fitting, and data printout. Multiple gamma-emitting radionuclide analysis in specific counting regions with automatic spill-down and spillover calculations is performed by the computer, including activity analysis calculations for dual or multiple radionuclide mixtures [55]. Some examples of multiple radionuclide isotope labels and applications carried out by solid scintillation gamma spectrometry are (a)  $^{125}\text{I}$ - $^{57}\text{Co}$  for the common folic acid-vitamin  $\text{B}_{12}$  assays in serum samples [55], (b)  $^{125}\text{I}$ - $^{131}\text{I}$  in the measurement of biological half-life [56], (c)  $^{57}\text{Co}$ - $^{58}\text{Co}$  used in the Schilling test to diagnose deficiencies in vitamin  $\text{B}_{12}$  absorption, (d)  $^{134}\text{Cs}$ - $^{137}\text{Cs}$  measurements in the environment, and (e) a six radionuclide mixture of  $^{125}\text{I}$ - $^{141}\text{Ce}$ - $^{51}\text{Cr}$ - $^{113}\text{Sn}$ - $^{103}\text{Ru}$ - $^{95}\text{Nb}$  for calculating regional blood flow with radionuclide-labelled microspheres [57].



*FIG. 13. Staggered array of ten NaI(Tl) scintillation detectors and photomultiplier tubes of a high-throughput automatic gamma analyser. On the right, two exposed photomultiplier tubes and NaI(Tl) detectors are illustrated. A see-through diagram of one of the NaI(Tl) detectors is at the far right with a sample tube in position for analysis. A lead counterweight and elevator are shown above and below the sample tube. Sample tubes in cassettes directly below the detectors are moved upward by elevators into counting position. (Courtesy of Packard Instrument Co., Meriden, CT 06450, USA.).*

## 8.2. Solid scintillation analysis in microplates

Solid scintillation counting of beta-particle and Auger-electron emitting radionuclides in microplates is of growing interest. This involves depositing the sample into the well of a special plastic microplate (e.g. LumaPlate), which contains a solid scintillator at the bottom of the well. The material is dried and then counted in the TopCount Microplate Scintillation Counter (Figs 8 and 9). By this technique, the activities (DPM) of small volumes of any nonvolatile samples labeled with beta particle- or Auger electron-emitting nuclides may be determined. The small samples involved are particularly applicable to cytotoxicity, immunoassay, receptor binding, enzyme activity, and other metabolic studies [19]. In certain circumstances the technique can replace conventional liquid scintillation analysis in counting vials when nonvolatile forms of the radionuclides and small volumes can be analysed. Typical optimum counting efficiencies (% E) achieved by the solid scintillation application for some common radionuclides deposited in the 96-well Lumaplate are  $^3\text{H}$  (49% E),  $^{14}\text{C}$  (85% E),  $^{32}\text{P}$  (87% E),  $^{51}\text{Cr}$  (24% E) and  $^{125}\text{I}$  (75% E) with background count rates of 8 to 9 CPM. The 24-well LumaPlate provides significantly higher counting efficiencies, i.e.  $^3\text{H}$  (55% E),  $^{14}\text{C}$  (95% E),  $^{32}\text{P}$  (93% E),  $^{51}\text{Cr}$  (48% E) and  $^{125}\text{I}$  (83% E), but with higher backgrounds of about 19 to 20 CPM.

## 8.3. Scintillation proximity assay

Scintillation proximity assay (SPA) is a modern radioisotope technology used for the analysis of binding reactions, commonly studied in agricultural biochemistry, molecular biology, and medical sciences; it circumvents the need to separate bound from free fractions. Yttrium silicate scintillating glass microspheres or plastic scintillator microspheres are used in this assay together with an isotope-labeled ( $^3\text{H}$  or  $^{125}\text{I}$ ) ligand. With the use of modern liquid-handling equipment and an automatic conventional liquid scintillation counter or microplate scintillation analyser for the counting of samples in a microplate format, hundreds of samples may be prepared and analysed in a single day, because traditional processes for the separation of bound and free fractions are not required. See Fig. 14 for an illustration of the basic principal. Thorough reviews of the principals and techniques of SPA are available in the literature [1, 55, 58, 59].

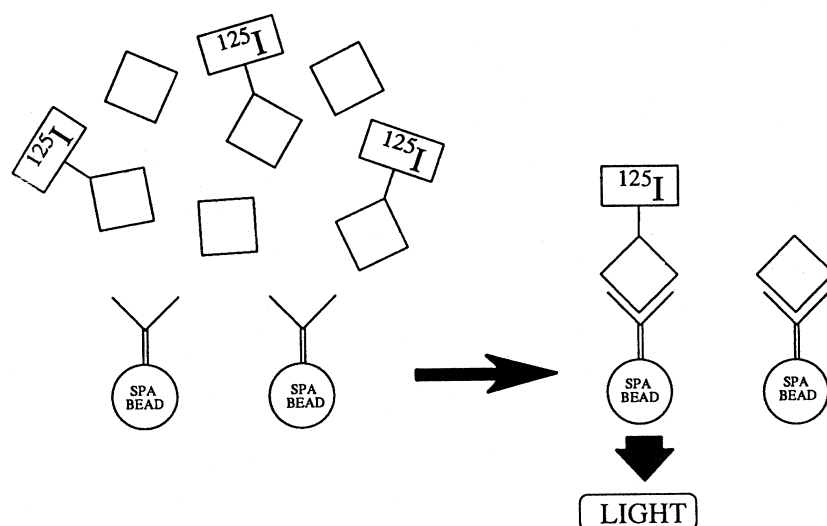


FIG. 14. Illustration of the principal of scintillation proximity assay (SPA). A microsphere scintillator (SPA bead) containing an attached antibody or receptor molecule binds with an  $^{125}\text{I}$ -labelled antigen or ligand molecule (a  $^3\text{H}$  label instead of  $^{125}\text{I}$  may also be used). The binding brings the  $^{125}\text{I}$ -nuclide label (or  $^3\text{H}$ ) in close proximity to the SPA bead. An Auger electron from the  $^{125}\text{I}$  (or beta particle from  $^3\text{H}$ ) on the bound antigen or ligand molecule can penetrate the SPA bead, producing scintillation with the emission of visible light in all directions. In contrast, unbound  $^{125}\text{I}$  (or  $^3\text{H}$ ) ligands or bound unlabeled ligands do not produce light emission. Ligands that compete for radionuclide-labeled ligand-binding sites reduce the detected light emission. (Adapted from Buchan et al. [60].)

Applications of SPA in agricultural and biological sciences include: (a) immunoassays [55, 61], (b) receptor-binding assays [55], and (c) enzyme assays [55]. Numerous kits for SPA are available commercially from Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA, and Amersham Pharmacia Biotech UK Ltd., Little Chalfont, Buckinghamshire, England. Scintillation proximity assays are not limited to applications with radionuclides emitting very low-energy particulate radiations, namely  $^3\text{H}$  ( $E_{\text{max}} = 18.6 \text{ keV}$ ) or  $^{125}\text{I}$  ( $E_{\text{av}} = 18 \text{ keV}$ ). However, the application of  $^{33}\text{P}$  ( $E_{\text{max}} = 249 \text{ keV}$ ) to SPA has recently been developed for specific assays such as high throughput kinase assays [62] and screening of kinase inhibitors [63]. Because of the relatively high energies of the beta particles emitted by  $^{33}\text{P}$  and the consequent relatively long length of travel of these particles, it is necessary to minimize scintillation proximity bead contact with free radionuclide label in the solution medium. This is accomplished by letting the beads settle overnight, microplate centrifugation, or suspension (flotation) of the scintillation proximity beads in 3 *M* caesium chloride [63].

## 9. RADIONUCLIDE STANDARDIZATION

One of the greatest advances in modern radioisotope analysis is the possibility of using a conventional liquid scintillation analyser and personal computer to standardize radionuclide samples, that is, determine the absolute activity of the sample with calculated uncertainties of often <1%. The method was developed as a result of close collaboration in research and development between the Centro de Investigaciones Energéticas Medioambientales y Tecnológicas (CIEMAT), Madrid, Spain, and the National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA. The CIEMAT/NIST radionuclide standardization method is dealt with in detail in a comprehensive text by Grau Malonda [64], who originally conceived of the method and who has continued to remain active in its development. Only a brief summary is provided here, together with some recent proposed modifications.

### 9.1. CIEMAT/NIST efficiency tracing with $^3\text{H}$

The CIEMAT/NIST method is based on the theoretical computer-based calculations of the counting efficiency of the radionuclide of interest as well as tritium for various values of the free parameter ( $\lambda$ ) and an experimental quench-correction curve obtained with a set of NIST-traceable quenched standards of  $^3\text{H}$ . The free parameter ( $\lambda$ ) is defined as the beta-particle energy in keV required to produce one photoelectron at the first dynode of the photomultiplier tube. The relationship of radionuclide counting efficiency ( $\varepsilon$ ) and free parameter for pure beta-emitters and liquid scintillation instruments consisting of two photomultiplier tubes in coincidence is defined by:

$$\varepsilon = \int_0^{E_{\text{max}}} N(E) \left\{ 1 - \exp \left[ -\frac{E \cdot X(E)}{2\lambda} \right] \right\}^2 dE \quad (9.1)$$

where

$E_{\text{max}}$  is the maximum beta-particle energy,

$N(E)$  is the theoretical beta-particle energy distribution,

$X(E)$  is the correction for ionization quenching and wall losses,

and  $\lambda$  is the free parameter [19, 64–68].

The term ‘free parameter’ was referred to as the ‘figure of merit’ in early papers on this method. It is now more commonly called the ‘free parameter’ to avoid confusion with the term ‘figure of merit’ used in the comparison of counting efficiencies and background count rates in low-level liquid scintillation analysis.

Computer programs have been developed for the rapid computation of counting efficiency as a function of free parameter [64, 69–75]. The procedure integrates theoretical calculations with experimental measurements. The free parameter is an essential component in the theoretical calculation of the counting efficiency; and the quench-indicating parameter (QIP) of a particular liquid scintillation analyser [e.g., tSIE, SQP(E), or H#] is required for the experimental aspect of the method. However, the free parameter cannot be obtained directly from experiment and the quenching

parameter is not theoretically computable [65]. Therefore, the method makes use of a plot that relates the free parameter with the QIP. Such a plot is obtained by integrating an experimental quench-correction curve of  $^3\text{H}$  (e.g., counting efficiency vs. a QIP) with a theoretically computed curve of counting efficiency versus free parameter. The CIEMAT/NIST procedure is outlined in Fig. 15. For more detailed treatments of the method, see Refs. [19, 64]. It is also referred to as the CIEMAT/NIST efficiency tracing method with  $^3\text{H}$ , but it should not be confused with the efficiency tracing direct DPM method described in Section 3.1. The latter method has no relation to the CIEMAT/NIST technique.

The CIEMAT/NIST method is currently popular for radionuclide standardization, as no special equipment is required. A common modern liquid scintillation analyser and personal computer are all that is needed. It has been employed for the standardization of a large number of radionuclide samples, some of which, to note only a few, are the following, with the calculated uncertainties in parenthesis and cited reference in brackets:  $^{14}\text{C}$  (0.2%) [67],  $^{36}\text{Cl}$  (0.3%) [76],  $^{63}\text{Ni}$  (0.1%) [77],  $^{35}\text{S}$  (1.0%) [78],  $^{54}\text{Mn}$  (0.71%) [79],  $^{93\text{m}}\text{Nb}$  (0.56%) [80],  $^{45}\text{Ca}$  (<0.25%) and  $^{55}\text{Fe}$  (<0.6%) [81],  $^{59}\text{Fe}$  (0.4%) and  $^{131}\text{I}$  (0.4%) [82],  $^{65}\text{Zn}$  (0.5%) [83],  $^{90}\text{Sr}$  (0.14%) [84] and  $^{110\text{m}}\text{Ag}$  (0.2%) [85].

## 9.2. Potential universal application

In the CIEMAT/NIST method, the counting efficiency as a function of free parameter ( $\lambda$ ) is found for both the standard nuclide  $^3\text{H}$  ( $\varepsilon_T$ ) and the radionuclide of interest ( $\varepsilon_{nuc}$ ). Under such circumstances, the ratio of the counting efficiencies ( $\varepsilon_T/\varepsilon_{nuc}$ ) is also known as a function of  $\lambda$  [66]. Consequently, as the counting efficiency of the  $^3\text{H}$  standard is known, the other can be computed.

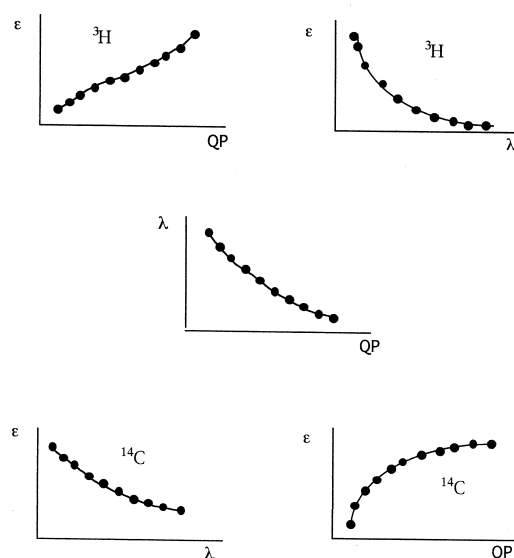


FIG. 15. Calculated and experimental calibration curves obtained in the radionuclide standardization method of CIEMAT/NIST efficiency tracing with  $^3\text{H}$ . The curves are described in the sequence in which they are obtained as follows: (upper left) experimental quench-correction curve of  $^3\text{H}$  counting efficiency versus quench-indicating parameter (QP); (upper right) computed  $^3\text{H}$  counting efficiency versus free parameter; (centre) “universal” curve of free parameter versus quench indicating parameter derived from the preceding two relationships; (lower left) computed curve of counting efficiency of the nuclide of interest (e.g.  $^{14}\text{C}$ ) versus the free parameter; and (lower right) quench curve of counting efficiency of the nuclide of interest (e.g.  $^{14}\text{C}$ ) versus the instrumental quench indicating parameter derived from the previous two curves. (From Grau Malonda [57].)



Tritium is considered, therefore, not only as a standard nuclide, but also as a tracer nuclide, because the counting efficiency of the nuclide of interest is found against that of the reference nuclide,  $^3\text{H}$  in this case. With this in mind and in view of the curves illustrated in Fig. 15, it was concluded that the counting efficiency of a given nuclide ( $\varepsilon_{nuc}$ ) at a certain QIP could be determined from the counting efficiency of tritium ( $\varepsilon_T$ ) at the same QIP. Therefore, to facilitate the application of the CIEMAT/NIST efficiency tracing method Günther [86] proposed the following three steps:

- **Calculation of the efficiencies.** The calculation of the efficiencies of radionuclides ( $\varepsilon_{nuc}$ ) versus the corresponding tritium efficiency ( $\varepsilon_T$ ) should be done firstly in a national standards laboratory. The results should then be parameterized as coefficients  $k_i$  of the polynomial in equation 9.2. The coefficients  $k_i$  of the least squares fit have been calculated at the Physikalisch-Technische Bundesanstalt (PTB), Braunschweig, Germany, for a large number of pure beta-emitters and beta-gamma emitters, many of which are used as tracers in the agricultural and biological sciences. The coefficients  $k_i$  have been tabulated and are available from the literature [19, 86].

$$\varepsilon_{nuc} = \sum_{i=0}^3 k_i \varepsilon_T^i \quad (9.2)$$

- **Verifying the results.** Verification of the calculated coefficients  $k_i$  for each radionuclide is best performed at a national standards laboratory with stocks of standard solutions and experimental comparison of results with those obtained from other methods of standardization. The PTB has demonstrated that the calculated coefficients, tabulated in the literature [1, 19, 86] are valid to within 0.5% uncertainty in most cases.
- **Laboratory procedure.** The user must determine the QIP [e.g. tSIE, SQP(E), or H#] automatically with each single measurement, determining the corresponding tritium efficiency ( $\varepsilon_T$ ) of the individual measurement using his or her own quench curve, and then calculating the efficiency of the radionuclide of interest ( $\varepsilon_{nuc}$ ) using the tritium efficiency and the tabulated coefficients for that nuclide [1, 19, 86]. A computer program can shorten the procedure. The tritium efficiency measurements obtained with the quench-correction curve of the liquid scintillation analyser, as well as the polynomial coefficients  $k_i$ , are included in the computer program (LSCAL, available from Günther [86] at the PTB) to obtain and use an efficiency curve with new coefficients  $m_i$  according to the equation:

$$\varepsilon_{nuc} = \sum_{i=0}^n m_i Q^i \quad (9.2.1)$$

With this novel procedure, summarized in Fig. 16, only the count rate (CPM) of the radionuclide sample and the quench-indicating parameter  $Q$  (same as the QIP such as, tSIE, SQP(E), or H#) are necessary to obtain the sample counting efficiency and activity (DPM). If the liquid scintillation analyser is equipped with a self-normalization procedure and evidenced stability with an automated instrument performance assessment (IPA), the repetition of the determination of the tritium quench-correction curve need not be carried out except over long intervals of time.

## 10. INSTRUMENT PERFORMANCE ASSESSMENT

During the pre-computer age of radioactivity analysis, it was a common practice to include controls each time experimental samples were counted or analysed. Such controls included standards of known activity of the radionuclide of interest and blank samples containing no added radioisotope. The objective was to check that the instrument was stable in terms of providing constant performance in the measurement of a standard within the acceptable deviations in accord with the random nature of nuclear decay. The blank sample provided a measure of the instrument stability in terms of background count rates.

## New calibration method

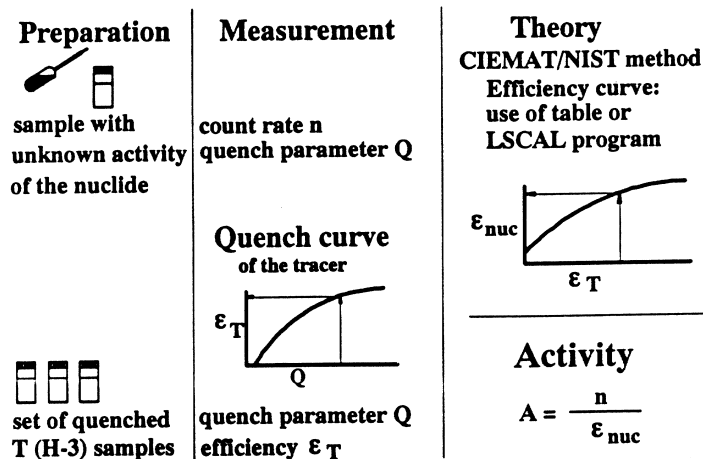


FIG. 16. The counting efficiency of the nuclide to be measured ( $\epsilon_{nuc}$ ) is obtained from the quench-indicating parameter ( $Q$ ) of the measurement, the tritium quench curve and the tabulated coefficients of an efficiency curve of the nuclide. The activity ( $A$ ) of the nuclide in DPM is calculated from its count rate ( $n$ ) and counting efficiency ( $\epsilon_{nuc}$ ). (From Günther [86].)

Modern instruments are computer-controlled, and instrument performance assessment (IPA) can be performed and recorded automatically by the computer. Assessing the performance of the instrument on a routine basis is important to provide assurance that the instrument is operating within acceptable parameters and to have a standing record over time of the instrument performance. Evidence of instrument performance recorded on a daily, weekly, or monthly basis can provide proof that deviations in instrument performance do not have any effect on the analytical results.

The IPA in modern liquid scintillation analysers are initiated automatically by the computer programs and the tests are carried out with NIST-traceable  $^3\text{H}$  and  $^{14}\text{C}$  unquenched standards as well as an unquenched blank sample for background measurements. In addition to an automatic instrument normalization and calibration, eight IPA parameters assessed with these standards are: (a) tritium efficiency, (b) tritium background, (c) tritium figure of merit, (d) tritium chi-square, (e)  $^{14}\text{C}$  efficiency, (f)  $^{14}\text{C}$  background, (g)  $^{14}\text{C}$  figure of merit, and (h)  $^{14}\text{C}$  chi-square. The results are stored and printed in a tabular and/or graphic form with a time and date stamp. The data points are stored in the memory and plotted. The graphs can be displayed, stored in the computer memory, and printed for each of the eight IPA parameters described previously. In any given graph, such as the example illustrated in Fig. 17, an average line is calculated automatically and the one, two, and three sigma values are provided so that the user can evaluate any trends or outlying points. When an outlining point is observed, the information is printed in the final data output. When parameters occur too frequently outside recommended limits, the instrument computer program should provide the user with a series of warnings or suggestions so that (s)he can assess the problem further and take preventative action [19].

Modern solid scintillation instruments are also equipped with similar IPA programs [55]. They differ only in the standards and sources used and the instrument parameters measured. Some standards used are  $^{129}\text{I}$  and  $^{137}\text{Cs}$  sources for detector calibration, chi-square, and resolution IPA tests,  $^{125}\text{I}$  standard for counting efficiency IPA data, and an empty sample tube for IPA background count rates. State-of-the-art flow scintillation analysis (FSA) instrumentation, which employs both liquid and solid scintillation detection methods to the quantitative analysis of radioisotopes in a flowing system such as high-performance liquid chromatography (HPLC) effluents, also perform IPA tests similar to those previously described [87].

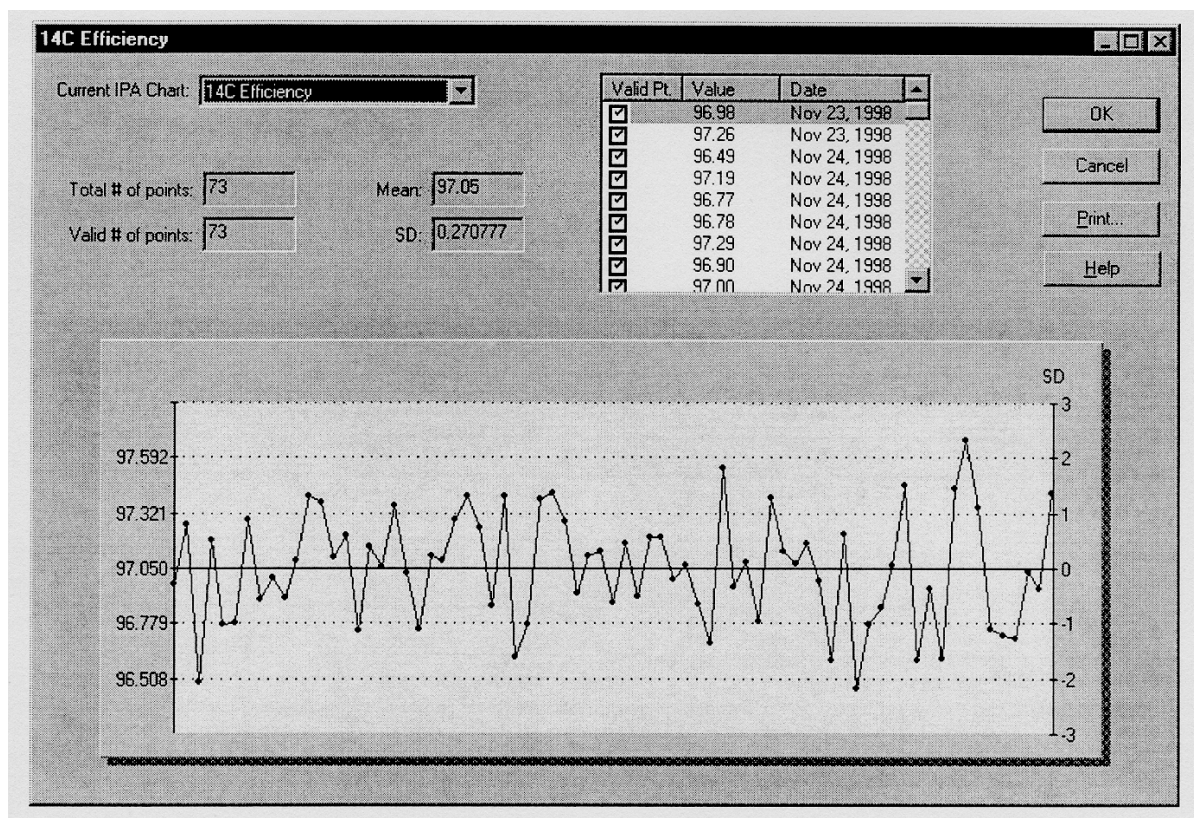


FIG. 17. Carbon-14 counting efficiency instrument performance assessment data displayed on a modern liquid scintillation analyser computer monitor (lower) as a graph with data points with an average base line and one, two, and three sigma values, and (upper) as a table listing  $^{14}\text{C}$  counting efficiency values and dates of measurement. (Courtesy of Packard Instrument Co., Meriden, CT 06450, USA.)

## 11. 'REPLAY'

In Section 2 it was noted that modern liquid scintillation analysers store the pulse height spectra of quenched standards on computer hard disk, which permit the automatic generation of any quench-correction curve of % E versus QIP regardless of the different lower level (LL) and upper level (UL) pulse height discriminator settings selected. State-of-the-art instruments also store automatically on computer hard disk all of the pulse height spectra of experimental samples counted in a given assay, regardless of the number of samples counted, even if thousands of samples are assayed. If, once samples are counted, it is decided that it would be best to assay the samples again under new counting conditions, would it be necessary to recount the samples? A modern instrument that can store the pulse height spectra of assayed samples obviates the need to recount samples in many circumstances. With stored sample pulse height spectra, the following changes can be made on a given assay without recounting the samples: (a) counting regions defined by LL and upper UL discriminator settings, (b) quench indicating parameter, (c) luminescence correction, (d) background subtraction, and (e) half-life correction. After modifying any of the aforementioned assay parameters, the instrument computer can generate new count rates (CPM) and disintegration rate (DPM) values of samples without recounting samples, often referred to as 'Replay.' The new sample data for thousands of samples are generated within seconds without recounting, and a hard copy of the results can be printed subsequently. The timesaving advantages of 'Replay' are immense. Also 'Replay' can be used to manually search for optimized regions in low-level liquid scintillation counting by modifying the LL and UL discriminators to generate new count rates without recounting samples.

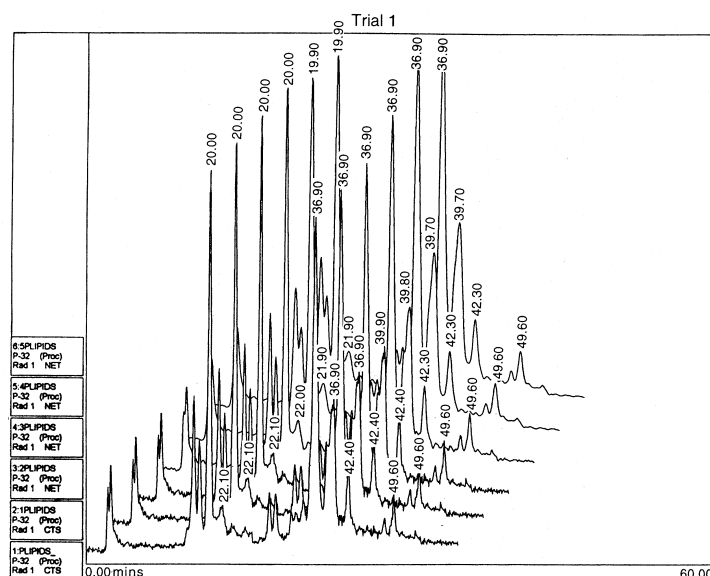


FIG. 18. Three-dimensional overlay of six flow scintillation analysis traces of HPLC separations of  $^{32}\text{P}$ -labeled lipids (radioactivity vs. retention time in minutes) with Packard FLO-ONE for Windows software. Up to thirty-two traces can be overlaid to compare differences or offset into a three-dimensional display as illustrated. From the display it is possible to analyse changes in data over a series of runs. (Courtesy of Packard Instrument Co., Meriden, CT, USA.)

## 12. FLOW SCINTILLATION ANALYSIS

Many modern advances have been made in the field of flow scintillation analysis (FSA), i.e. the application of liquid or solid scintillation detection methods to the quantitative analysis of radionuclide activity in a flowing system. The technology is currently applied most commonly to the measurement of radioisotope tracers in high-performance liquid chromatography (HPLC) effluent streams, referred to as radio-HPLC. The applications of radio-HPLC are now widespread in many fields of science, including plant and soil biochemistry, agricultural and food chemistry, general biochemistry, molecular biology, and associated fields such as medical and drug research [87]. These fields often require the use of HPLC to separate molecular compounds and, when radionuclides are used to study the metabolic fate of compounds, radio-HPLC is now often the method of choice to separate and quantify the activity levels of radionuclides in the molecular structures.

Modern flow scintillation analysers are operated by a computer equipped with multitasking software including automatic quench correction and detection-efficiency determination, DPM measurements in the effluent stream, background reduction by time-resolved liquid scintillation counting [88], multichannel analysis and spectral display, self-normalization and calibration, pulse height spectral display, preset and variable energy regions in keV for activity analysis, dual independent counting regions for either automatic single- or dual-radionuclide analysis with update times from 1 to 120 s, software for radio-HPLC workstation direct instrument control and data reduction including three-dimensional and overlay display of activity peaks from different chromatogram traces as illustrated in Fig. 18, and instrument performance assessment (IPA).

An important advance in FSA is the manufacture of the relatively new heterogeneous flow cell containing monocrystalline SolarScint™ (trademark of Packard Instrument Company, Meriden, CT, USA). SolarScint™ undergoes minimal compound binding when used in the pH range of 3 to 8 providing optimal HPLC peak resolutions and detection efficiencies of 3.0% and 70% for  $^3\text{H}$  and  $^{14}\text{C}$ , respectively. Homogeneous liquid cells provide higher detection efficiencies (i.e. 20–60% for  $^3\text{H}$  and 70–95% for  $^{14}\text{C}$ ); however, they require a stream splitter to divert part of the HPLC effluent to a fraction collector for compound isolation, or to a mass spectrometer (MS) or nuclear magnetic

resonance (NMR) spectrometer for compound structural elucidation. The SolarScint™ solid heterogeneous cell permits the entire effluent stream to continue on after radioactivity analysis to the fraction collector or MS and NMR spectrometer. This is often vital, because of the small amounts ( $\mu\text{g}$ ) of compounds isolated via the HPLC.

The most modern advance of radio-HPLC is the direct linking of the flow scintillation analyser to the mass spectrometer or the NMR spectrometer for compound structure identification. The use of radio-chromatography for compound radioactivity analysis and isolation followed by mass spectrometry and NMR spectrometry for compound structure are not new to the field of soil biochemistry [26, 89–95] or to the biological sciences in general. However, the cutting edge in this technology today is to eliminate the tedious step of compound isolation by linking the HPLC, via the Packard Radiomatic flow scintillation analyser, directly to the mass spectrometer [96–99] or NMR spectrometer [100–102]. The possibility of determining molecular structure of radioisotope-labelled compounds directly in the HPLC effluent without the need of compound isolation provides immense savings in time and effort. In the not-too-distant future, numerous research papers in the biological sciences, including soil and agricultural chemistry, will report the use of flow scintillation analysis with HPLC and mass spectrometry and NMR spectrometry.

With specific reference to applications in the agricultural and biological sciences, future trends in the measurement of radioisotope activity are likely to be in the further development of computer-based instrumentation that will provide (a) lower background count rates, (b) higher sample throughput, (c) automated analyses of several radionuclides in mixtures, and (d) new absolute activity (DPM) methods through more sophisticated computer programs and calculations.

## REFERENCES

- [1] L'ANNUNZIATA, M.F., (Ed.), Handbook of Radioactivity Analysis. Academic Press Inc., San Diego (1998). ISBN: 0-12-436255-9.
- [2] L'ANNUNZIATA, M.F., Efficiency Tracing DPM and Direct DPM—Instrument Performance Data, Counter Intelligence Tri-Carb Application Note 004, Packard Instrument Co., Meriden, CT (1997).
- [3] TAKIUE, M., ISHIKAWA, H., Thermal neutron reaction cross section measurements for fourteen nuclides with a liquid scintillation spectrometer, Nucl. Instrum. Methods **148** (1978) 157–161.
- [4] ISHIKAWA, H., et al., Radioassay by an efficiency tracing technique using a liquid scintillation counter, Int. J. Appl. Radiat. Isot. **35** (1984) 463–466.
- [5] FUJII, H., TAKIUE, M., Radioassay of dual-labeled samples by sequential Cherenkov counting and liquid scintillation efficiency tracing technique, Nucl. Instrum. Methods Phys. Res. Sect. A **273** (1988) 377–380.
- [6] FUJII, H., TAKIUE, M., Radioassay of alpha- and beta-emitters by sequential Cherenkov and liquid scintillation countings, Appl. Radiat. Isot. **39** (1988) 327–330.
- [7] NOOR, A., et al., Cerenkov and liquid scintillation analysis of the triple label  $^{86}\text{Rb}$ - $^{35}\text{S}$ - $^{33}\text{P}$ , Appl. Radiat. Isot. **47** (1996) 659–668.
- [8] MENZEL, R.G., SMITH, S.J., “Soil fertility and plant nutrition”, Isotopes and Radiation in Agricultural Sciences, Vol. 1, Soil-Plant-Water Relationships, (L'ANNUNZIATA, M.F., LEGG, J.O., Eds.), Academic Press, London (1984) 1–34.
- [9] HOMMA, Y., et al., “Comparison of a modified integral counting method and efficiency tracing method for the determination of  $^{222}\text{Rn}$  by liquid scintillation counting”, Liquid Scintillation Spectrometry 1992 (NOAKES, J.E., et al., Eds.), Radiocarbon, Tucson (1993) 59–62.
- [10] HOMMA, Y., et al., “Determination of atmospheric radioactivity using a membrane filter and liquid scintillation spectrometry”, Liquid Scintillation Spectrometry 1992 (NOAKES, J.E., et al., Eds.), Radiocarbon, Tucson (1993) 63–67.
- [11] HOMMA, Y., et al., The zero detection threshold of a liquid scintillation spectrometer and its application to liquid scintillation counting, Appl. Radiat. Isot. **45** (1994) 341–344.

- [12] HOMMA, et al., Absolute liquid scintillation counting of  $^{35}\text{S}$  and  $^{45}\text{Ca}$  using a modified integral counting method, *J. Radioanal. Nucl. Chem. Letts.* **187** (1994) 367–374.
- [13] HOMMA, Y., et al., A modified integral counting method and efficiency tracing method for measuring  $^{222}\text{Rn}$  by liquid scintillation counting, *Appl. Radiat. Isot.* **45** (1994) 699–702.
- [14] MURASE, Y., et al., Assay of  $^{222}\text{Rn}$  in water samples by a modified integral counting method, *Appl. Radiat. Isot.* **49** (1998) 861–865.
- [15] PASSO Jr., C.J., COOK, G.T., *Handbook of Environmental Liquid Scintillation Spectrometry a Compilation of Theory and Methods*, Publ. No. PMC0387, Packard Instrument Co., Meriden, CT (1994).
- [16] COOK, G.T., et al., “Environmental Liquid Scintillation Analysis”, *Handbook of Radioactivity Analysis* (L’ANNUNZIATA, M.F., Ed.), Academic Press, San Diego (1998) 331–386.
- [17] PASSO Jr., C.J., KESSLER, M.J., “Selectable delay before burst—a novel feature to enhance low-level counting performance”, *Liquid Scintillation Spectrometry 1992*, (NOAKES, J.E., et al., Eds.), Radiocarbon, Tucson (1993) 51–57.
- [18] PASSO Jr., C.J., ROBERTS, D.J., “Expanded energy for time-resolved liquid scintillation counting: An enhancement for programmable TR-LSC<sup>®</sup>”, *Liquid Scintillation Spectrometry 1994* (COOK, G.T., et al., Eds.), Radiocarbon, Tucson (1996) 67–74.
- [19] L’ANNUNZIATA, M.F., KESSLER, M.J., “Radiotracer liquid scintillation analysis”, *Handbook of Radioactivity Analysis* (L’ANNUNZIATA, M.F., Ed.), Academic Press, San Diego (1998) 209–329.
- [20] LADD, J.N., MARTIN, J.K., “Soil organic matter studies”, *Isotopes and Radiation in Agricultural Sciences, Vol. 1, Soil-Plant-Water Relationships* (L’ANNUNZIATA, M.F., LEGG, J.O., Eds.), Academic Press, London (1984) 67–98.
- [21] NIELSEN, D.R., CASSEL, D.K., “Soil water management”, *Isotopes and Radiation in Agricultural Sciences, Vol. 1, Soil-Plant-Water Relationships* (L’ANNUNZIATA, M.F., LEGG, J.O., Eds.), Academic Press, London (1984) 35–65.
- [22] L’ANNUNZIATA, M.F., Cerenkov Counting of  $^{32}\text{P}$ —Instrument Performance Data, Counter Intelligence Tri-Carb Application Note 002, Packard Instrument Co., Meriden, CT (1997).
- [23] L’ANNUNZIATA, M.F., “Cherenkov counting”, *Handbook of Radioactivity Analysis* (L’ANNUNZIATA, M.F., Ed.), Academic Press, San Diego (1998) 453–505.
- [24] PRICHARD, H.M., et al., Liquid scintillation analysis of  $^{222}\text{Rn}$  in water by alpha-beta discrimination, *Radioact. Radiochem.* **3** (1992) 28–36.
- [25] EFFERTZ, B., et al., “Single photomultiplier technology for scintillation counting in microplates”, *Liquid Scintillation Spectrometry 1992* (NOAKES, J.E., et al., Eds.), Radiocarbon, Tucson (1993) 37–42.
- [26] L’ANNUNZIATA, M.F., “Agricultural biochemistry: reaction mechanisms and pathways in biosynthesis”, *Isotopes and Radiation in Agricultural Sciences, Vol. 2, Animals, Plants, Food and the Environment* (L’ANNUNZIATA, M.F., LEGG, J.O., Eds.), Academic Press, London (1984) 105–182.
- [27] SCHNEIDER, E.W., VERBRUGGE, M.W., Radiotracer method for simultaneous measurement of cation, anion and water transport through ion exchange membranes, *Appl. Radiat. Isot.* **44** (1993) 1399–1408.
- [28] L’ANNUNZIATA, M.F., *Radionuclide Tracers, Their Detection and Measurement*, Academic Press Inc., San Diego (1987).
- [29] L’ANNUNZIATA, M.F., Comparison of Conventional and Full Spectrum DPM (FS-DPM). Analysis of  $^{33}\text{P}$ - $^{32}\text{P}$  Double Labels—Instrument Performance Data, Counter Intelligence Tri-Carb Application Note 003, Packard Instrument Co., Meriden, CT (1997).
- [30] NOOR, A., et al., Application of pulse height spectral analysis to double-label counting of  $^{35}\text{S}$ - $^{32}\text{P}$ , *Appl. Radiat. Isot.* **46** (1995) 791–797.
- [31] NOOR, A., et al., Cherenkov and liquid scintillation analysis of the triple label  $^{86}\text{Rb}$ - $^{35}\text{S}$ - $^{33}\text{P}$ , *Appl. Radiat. Isot.* **47** (1996) 767–775.
- [32] KESSLER, M.J., (Ed.), *Liquid Scintillation Analysis, Science and Technology*, Publ. No. 169-3052, Packard Instrument Co., Meriden, CT (1989).
- [33] DE FILIPPIS, S.,  $^{55}\text{Fe}$  and  $^{59}\text{Fe}$ : A quantitative comparison of four methods of liquid scintillation activity analysis, *Radioact. Radiochem.* **2** (1991) 14–21.

- [34] VAN CUATER, S., ROESSLER, N., "Modern techniques for quench correction and DPM determination in windowless liquid scintillation counting: A critical review", *Liquid Scintillation Counting and Organic Scintillators* (ROSS, H., Eds.), Lewis Publishers, Chelsea, MI (1991) 219–237.
- [35] TAKIUE, M., et al., A new approach to analytical radioassay of multiple beta-labeled samples using a liquid scintillation spectrometer, *Nucl. Instrum. Methods Phys. Res. Sect. A* **293** (1990) 596–600.
- [36] TAKIUE, M., et al., Liquid scintillation radioassay for multiple radionuclide mixtures by the most probable value theory, *J. Radioanal. Nucl. Chem.* **152** (1991) 227–236.
- [37] TAKIUE, M., et al., Analytical measurements of multiple beta-emitter mixtures with a liquid scintillation spectrometer, *J. Radioanal. Nucl. Chem. Lett.* **155** (1991) 183–193.
- [38] TAKIUE, M., et al., Nuclide identification of pure-beta emitter mixtures with liquid scintillation spectrometry, *Appl. Radiat. Isot.* **43** (1992) 853–857.
- [39] TAKIUE, M., et al., Liquid scintillation radioassay for low-activity beta-emitter mixtures by the method of least squares, *J. Radioanal. Nucl. Chem. Lett.* **200** (1995) 247–258.
- [40] MATSUI, Y., TAKIUE, M., Liquid scintillation radioassay of multi-labeled beta-emitters, *Appl. Radiat. Isot.* **42** (1991) 841–845.
- [41] GRAU CARLES, A., A new linear spectrum unfolding method applied to radionuclide mixtures in liquid scintillation spectrometry, *Appl. Radiat. Isot.* **45** (1993) 83–90.
- [42] GRAU CARLES, A., et al., Standardization of multi-nuclide mixtures by a new spectrum unfolding method, *J. Radioanal. Nucl. Chem. Lett.* **176** (1993) 391–403.
- [43] GRAU MALONDA, A., et al., Radioactivity determination of  $^{90}\text{Y}$ ,  $^{90}\text{Sr}$  and  $^{89}\text{Sr}$  mixtures by spectral deconvolution, *Nucl. Instrum. Methods Phys. Res. Sect. A* **339** (1994) 31–37.
- [44] GRAU CARLES, A., MLOG, the simultaneous standardization of multi-nuclide mixtures, *Comput. Phys. Commun.* **93** (1996) 48–52.
- [45] VERREZEN, F., HURTZEN, C., Radioassay of low-level, low-energy beta activity in multilabeled samples containing high-energy beta impurities using liquid scintillation spectrometry, *Radiocarbon*, Tucson (1996) 381–389.
- [46] VERREZEN, F., HURTZEN, C., A multiple window deconvolution technique for measuring low-energy beta activity in samples contaminated with high-energy beta impurities using liquid scintillation spectrometry, *Appl. Radiat. Isot.* **53** (2000) 289–296.
- [47] RUNDT, K., KOURU, H., Liquid Scintillation Counter for Measuring the Activity of Radioactive Samples Containing a Multiple of Radioactive Isotopes, US Patent No. 4,918,310 (1989).
- [48] RUNDT, K., KOURU, H., Apparatus and a Method for Measuring the Activity of Radioactive Samples Containing a Multiple of Radioactive Isotopes, US Patent No. 5,134,294 (1992).
- [49] BELANOV, S.V., KASHIREN, I.A., et al., The Method of Identifying Radionuclides with the Use of a Liquid Scintillation Counter, RF Patent No. 2,120,646 (1997).
- [50] BELANOV, S.V., KASHIREN, I.A., et al., The method of identifying radionuclides with the use of a liquid scintillation counter, RF Patent No. 98,106,407 (1998).
- [51] KASHIREN, I.A., ERMAKOV, A.I., et al., Liquid scintillation determination of low-level components in complex mixtures of radionuclides, *Appl. Radiat. Isot.* **53** (2000) 303–308.
- [52] GRAU CARLES, A., et al., Standardization of  $\text{U}(\text{X}_1 + \text{X}_2)$ : The  $^{234}\text{Th} + ^{234\text{m}}\text{Pa} + ^{230}\text{Th}$  mixture, *Nucl. Instrum. Methods Phys. Res. Sect. A* **369** (1996) 431–436.
- [53] TORIBO, M., et al., Simultaneous determination of plutonium alpha emitters by liquid scintillation counting using multivariate calibration, *Anal. Chim. Acta* **310** (1995) 297–305.
- [54] TORIBO, M., et al., "Optimization of counting conditions and simultaneous determination of  $^{238}\text{Pu}$ ,  $^{239}\text{Pu}$  and  $^{240}\text{Pu}$  by liquid scintillation counting", *Liquid Scintillation Spectrometry 1994* (COOK, G.T., Eds.), *Radiocarbon*, Tucson (1996) 157–165.
- [55] L'ANNUNZIATA, M.F., "Solid scintillation analysis", *Handbook of Radioactivity Analysis* (L'ANNUNZIATA, M.F., Ed.), Academic Press, San Diego (1998) 507–599.
- [56] NOZAKI, T., SAITO, J., Isotopic double tracers for the measurement of the biological half-life of an element or compound, exemplified by  $^{125}\text{I}$ - $^{131}\text{I}$  taken up in seaweed, *Appl. Radiat. Isot.* **46** (1995) 1299–1305.

- [57] ANONYMOUS, PCGERDA: A new software package for calculating regional blood flow using the radiolabeled microsphere technique, Gamma Notes, GN007, Packard Instrument Co., Meriden, CT (1993).
- [58] COOK, N.D., Scintillation proximity assay: A versatile high throughput screening technology, *Drug Discov. Today* **1** (1996) 287–294.
- [59] JESSOP, R.A., “New applications of scintillation proximity assay”, *Liquid Scintillation Spectrometry 1994* (COOK, G.T., et al., Eds.), Radiocarbon, Tucson (1996) 175–182.
- [60] BUCHAN, K.W., et al., Human placental membranes contain predominantly ET<sub>B</sub> receptors, *J. Cardiovasc. Pharmacol.* **22** (1993) S136–S139.
- [61] HORTON, J.K., BAXENDALE, P.M., “Mass measurements of cyclic AMP formation by radioimmunoassay, enzyme immunoassay, and scintillation proximity assay”, *Methods in Molecular Biology*, Vol. 41, Signal Transduction Protocols (KENDALL, D.A., HILL, S.J., Eds.), Humana Press, Totowa, NJ (1995) 91–105.
- [62] ANONYMOUS, Biodirectory 2000, Amersham Pharmacia Biotech Inc., Piscataway, NJ (2000).
- [63] PARK, Y.-W., et al., Optimization of <sup>33</sup>P scintillation proximity assays using cesium chloride bead suspension, *TopCount Topics*, TCA-030, Packard Instrument Co., Meriden, CT (1998).
- [64] GRAU MALONDA, A., *Free Parameter Models In Liquid Scintillation Counting*, Editorial CIEMAT, Madrid, ISBN: 84-7834-350-4 (1999).
- [65] GRAU MALONDA, A., GARCIA-TORAÑO, E., Evaluation of counting efficiency in liquid scintillation counting of pure beta-emitters, *Int. J. Appl. Radiat. Isot.* **33** (1982) 249–253.
- [66] GRAU MALONDA, A., et al., Liquid-scintillation counting efficiency as a function of the figure of merit for pure beta-particle emitters, *Int. J. Appl. Radiat. Isot.* **36** (1985) 157–158.
- [67] COURSEY, B.M., et al., Standardization of carbon-14 by  $4\pi\beta$  liquid scintillation efficiency tracing with hydrogen-3, *Appl. Radiat. Isot.* **37** (1986) 403–408.
- [68] COURSEY, B.M., et al., The standardisation of plutonium-241 and nickel-63, *Nucl. Instrum Methods Phys. Res. Sect. A* **279** (1989) 603–610.
- [69] GRAU MALONDA, A., et al., Free parameter codes to compute the counting efficiency in liquid scintillators, *Trans. Am. Nucl. Soc.* **55** (1987) 55–56.
- [70] GARCIA-TORAÑO, E., GRAU MALONDA, A., EFFY, a program to calculate the counting efficiency of beta particles in liquid scintillators, *Comput. Phys. Commun.* **23** (1981) 385–391.
- [71] GARCIA-TORAÑO, E., GRAU MALONDA, A., EFFY2, a new program to compute the counting efficiency of beta particles in liquid scintillators, *Comput. Phys. Commun.* **36** (1985) 307–312.
- [72] GARCIA-TORAÑO, E., GRAU MALONDA, A., EFYGA, a Monte Carlo program to compute the interaction probability and the counting efficiency of gamma rays in liquid scintillators, *Comput. Phys. Commun.* **47** (1987) 341–347.
- [73] GARCIA-TORAÑO, E., et al., EBEGA—The counting efficiency of a beta-gamma emitter in liquid scintillators, *Comput. Phys. Commun.* **50** (1988) 313–319.
- [74] LOS ARCOS, J.M., et al., VIASKL: A computer program to evaluate the liquid scintillation counting efficiency and its associated uncertainty for K-L-atomic shell electron-capture nuclides, *Comput. Phys. Commun.* **44** (1987) 209–220.
- [75] GRAU CARLES, A., et al., EMI, the counting efficiency for electron capture, electron capture-gamma and isomeric transition, *Comput. Phys. Commun.* **79** (1994) 115–123.
- [76] RODRIQUEZ BARQUERO, L., et al., Preparación y calibración por centelleo líquido de una muestra de Cl-36, *An. Fis. B* **85** (1989) 55–69.
- [77] ZIMMERMAN, B.E., COLLÉ, R., Cocktail volume effects in  $4\pi\beta$  liquid scintillation spectrometry with <sup>3</sup>H-standard efficiency tracing for low-energy beta-emitting radionuclides, *Appl. Radiat. Isot.* **48** (1997) 365–378.
- [78] CALHOUN, J.M., et al., “The standardization of <sup>35</sup>S methionine by liquid scintillation efficiency tracing with <sup>3</sup>H”, *Liquid Scintillation Counting and Organic Scintillators* (ROSS, H., Eds.), Lewis Publishers, Chelsea, MI (1991) 317–323.
- [79] RODRIQUEZ, L., et al., LSC standardization of <sup>54</sup>Mn in inorganic and organic samples by the CIEMAT/NIST efficiency tracing method, *Nucl. Instrument, Methods Phys. Res. Sect. A* **312** (1992) 124–131.



- [80] GÜNTHER, E., SCHÖTZIG, U., Activity determination of  $^{93m}\text{Nb}$ , Nucl. Instrument. Methods Phys. Res. Sect. A **312** (1992) 132–135.
- [81] ORTIZ, J.F., et al., “CIEMAT/NIST standardization method extended to anode outputs for beta and electron-capture nuclides”, Liquid Scintillation Spectrometry 1992 (NOAKES, J.E., et al., Eds.) Radiocarbon, Tucson (1993) 261–267.
- [82] GÜNTHER, E.W., Standardization of  $^{59}\text{Fe}$  and  $^{131}\text{I}$  by liquid scintillation counting, Nucl. Instrument. Methods Phys. Res. Sect. A **339** (1994) 402–407.
- [83] GÜNTHER, E., Standardization of the EC nuclides  $^{55}\text{Fe}$  and  $^{65}\text{Zn}$  with the CIEMAT/NIST tracer method, Appl. Radiat. Isot. **49** (1998) 1055–1060.
- [84] CECCATELLI, A., DE FELICE, P., Standardization of  $^{90}\text{Sr}$ ,  $^{63}\text{Ni}$  and  $^{55}\text{Fe}$  by the  $4\pi\beta$  liquid scintillation spectrometry method with 3H-standard efficiency tracing, Appl. Radiat. Isot. **51** (1999) 85–92.
- [85] GARCIA-TORAÑO, E., et al., Standardization of  $^{110m}\text{Ag}$  by liquid scintillation and  $4\pi\beta$ - $\gamma$  coincidence counting, Appl. Radiat. Isot. **52** (2000) 637–641.
- [86] GÜNTHER, E.W., “A simple method for transferring the tritium calibration of an LSC system to other radionuclides”, Liquid Scintillation Spectrometry 1994 (COOK, G.T., et al., Eds.), Radiocarbon, Tucson (1996) 373–379.
- [87] L’ANNUNZIATA, M.F., “Flow scintillation analysis”, Handbook of Radioactivity Analysis (L’ANNUNZIATA, M.F., Ed.) Academic Press, San Diego (1998) 601–646.
- [88] ANONYMOUS, New Technology Improves Radio-HPLC Detection, FSA Application Note, FSA-002, Packard Instrument Co., Meriden, CT (1996).
- [89] L’ANNUNZIATA, M.F., FULLER, W.H., Soil and plant relationships of inositol phosphate stereoisomers; the identification of D-chiro- and muco-inositol phosphates in a desert soil and plant system, Soil Sci. Soc. Amer. Proc. **35** (1971) 587–595.
- [90] L’ANNUNZIATA, M.F., FULLER, W.H., Nuclear magnetic resonance spectra of acetate derivatives of soil and plant inositol phosphates, Soil Sci. Soc. Amer. Proc. **35** (1971) 655–658.
- [91] L’ANNUNZIATA, M.F., et al., D-chiro-inositol phosphate in a forest soil, Soil Sci. Soc. Amer. Proc. **36** (1972) 183–184.
- [92] L’ANNUNZIATA, M.F., The origin and transformations of the soil inositol phosphate isomers, Soil Sci. Soc. Amer. Proc. **39** (1975) 377–379.
- [93] L’ANNUNZIATA, M.F., FULLER, W.H., Evaluation of the mass spectral analysis of soil inositol, inositol phosphates, and related compounds, Soil Sci. Soc. Amer. J. **40** (1976) 672–678.
- [94] L’ANNUNZIATA, M.F., GONZALEZ-ITURBE, J., “Soil metabolic transformations of carbon-14-myo-inositol, carbon-14-phytic acid and carbon-14-iron(III) phytate,” Soil Organic Matter Studies, Vol. I, IAEA, Vienna (1977) 239–253.
- [95] L’ANNUNZIATA, M.F., et al., Microbial epimerization of myo-inositol to chiro-inositol in soil, Soil Sci. Soc. Amer. J. **41** (1977) 733–736.
- [96] NOORT, D., et al., Alkylation of human serum albumin by sulfur mustard in vitro and in vivo: mass spectrometric analysis of a cysteine adduct as a sensitive biomarker of exposure, Chem. Res. Toxicol. **12** (1999) 715–721.
- [97] ADAS, F., et al., Involvement of cytochrome P450 2E1 in the ( $\omega$ -1)-hydroxylation of oleic acid in human and rat liver microsomes, J. Lipid Res. **39** (1998) 1210–1219.
- [98] KUMAR, G.N., et al., In vitro metabolism of the HIV-1 protease inhibitor ABT-378: species comparison and metabolite identification, Drug Metab. Dispos. **27** (1999) 86–91.
- [99] RISK, P., et al., Disposition and biotransformation of the antiretroviral drug nevirapine in humans, Drug Metab. Dispos. **27** (1999) 895–901.
- [100] BEERY, J.W., Personal Communication, BASF Corporation, Agricultural Research Center, Research Triangle Park, NC 27709 (2000) August 21.
- [101] BOWERS, G., Personal Communication, GlaxoWellcome Research and Development, Research Triangle Park, NC 27709 (2000) August 21.
- [102] SILVA-ELIPE, M.V., Personal Communication, Merck & Co., Inc., Drug Metabolism, Rahway, NJ 07065 (2000) August 22.

## Keynote Address

### TRENDS IN STABLE ISOTOPIC ANALYSES AND APPLICATIONS

J. OESSELMANN, A. HILKERT, C.B. DOUTHITT  
Finnigan MAT GmbH,  
Bremen, Germany

#### Abstract

Continuous-flow isotope ratio mass spectrometry (CF-IRMS) methodology is currently driving significant evolution in sample-preparation hardware for the determination of  $^{13}\text{C}$ ,  $^{15}\text{N}$ ,  $^{18}\text{O}$ ,  $^{34}\text{S}$ , and D in organic and inorganic samples. Currently, >50% of all new IRMS systems are bought without a dual viscous flow inlet system, and are used exclusively in continuous-flow mode. Samples analysed by continuous-flow methodologies include a wide variety of solids, liquids, and gases, in which the concentration of the analyte of interest varies over a very large dynamic range. The analyte must still be converted into a clean, pure gas prior to introduction into the ion source of the IRMS, but the “clean” gas is then a trace component in a stream of helium. The evolution of the practice of CF-IRMS is reviewed.

#### 1. INTRODUCTION

Between 1950, when the dual viscous-flow inlet system was introduced, and the mid-1980s, only minor modifications were made to the hardware bequeathed to the scientific community by Al Nier. It was the mating of capillary gas chromatography (GC) with IRMS that allowed the dual inlet system to be bypassed, and the new arrangement has been widely referred to as “continuous-flow IRMS,” because a carrier gas was used to achieve viscous flow conditions. Continuous-flow IRMS methodology has led to significant evolution in sample preparation hardware for the determination of  $^{13}\text{C}$ ,  $^{15}\text{N}$ ,  $^{18}\text{O}$ ,  $^{34}\text{S}$ , and D in organic and inorganic samples. The samples to be analysed include a wide variety of solids, liquids, and gases, in which the concentration of the analyte of interest may vary over a very large dynamic range. It is still required that the analyte be converted into a clean and pure gas prior to introduction into the ion source of the isotope ratio mass spectrometer, but this “clean” gas is now a trace component in a stream of helium gas. Figure 1 gives an overview of the CF-IRMS modules and current applications.

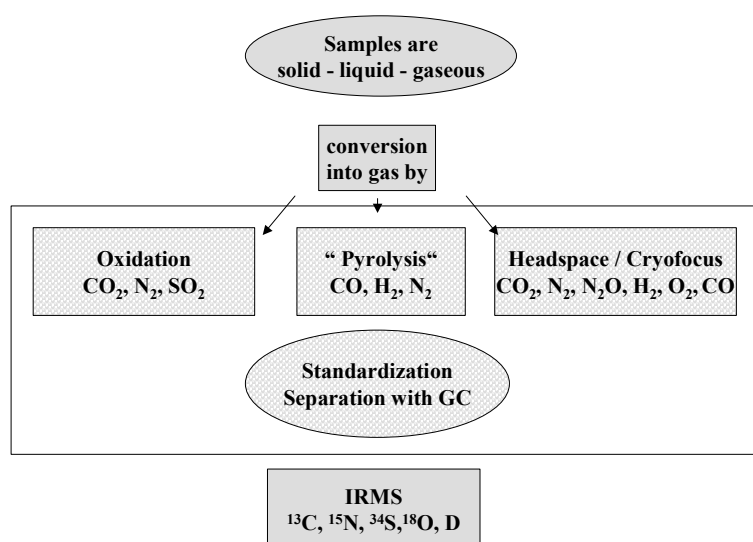


FIG. 1. Overview of continuous-flow techniques.

Not only the sample inlets have evolved; significant advances in collector technology (Fig. 2) allow novel measurement strategies including two components,  $\text{N}_2$  and  $\text{CO}_2$ , from a single combustion (Fig. 2b);  $\text{CO}$  and  $\text{H}_2$  from a high temperature carbon reduction; accurate and precise measurement of  $\text{D}/\text{H}$  of  $\text{H}_2$  in the presence of  $\text{He}$ , which requires an energy filter to reduce the tailing from  $4\text{He}^+$  (Fig. 2a, c); high-precision measurements of  $^{17}\text{O}/^{16}\text{O}$ , required to confirm existence of non-mass dependent fractionation (Fig. 2b); and direct measurements of the molecular ratios of the major atmospheric gas species (e.g.  $\text{N}_2/\text{O}_2/\text{Ar}$ , Fig. 2d).

The development of CF-IRMS applications and instruments has proceeded along several distinct and independent paths, with the two principal lines of descent being elemental analyser-IRMS and capillary GC-IRMS. The recognition that the standard sampling techniques used with capillary GCs (e.g. cryofocusing, loop sampling) could be adapted to isotope ratio measurements has allowed the practice of continuous flow to be extended to measurement of trace gases in the atmosphere, dissolved components, and analysis of waters and carbonates. We review the origins and current status of CF-IRMS and developments that are expected in the near future.

## 2. SOLID MATERIALS

The first known attempt to use an elemental analyser with a mass spectrometer to measure phytoplankton uptake of enriched nitrogen dates back to 1965 [1], when a group in the United States modified a Coleman nitrogen analyser to combust the sample and  $\text{CO}_2$  was used to sweep the nitrogen through the system. The  $\text{CO}_2$  was absorbed in  $\text{KOH}$  solution and the  $\text{N}_2$  cleaned over  $\text{LN}_2$  and fed into a Bendix time-of-flight machine. It is noteworthy that this apparatus was even taken to sea. In 1983, two hybrid systems were constructed, with a Japanese group using a small quadrupole mass spectrometer with a modified carbon-nitrogen elemental analyser [2], and an English group reporting on interfacing an automatic Carlo Erba elemental analyser with an isotope ratio mass spectrometer [3]. This last group can be said to have invented the practice of “continuous flow-IRMS,” which has led to the commissioning of over 600 elemental analyser (EA-IRMS) systems worldwide. This approach, which was originally focused on measurement of  $^{15}\text{N}$ , was rapidly extended to include  $^{13}\text{C}$  and  $^{34}\text{S}$  [4], and more recently to  $^{18}\text{O}$  and  $\text{D}$  [5].

### 2.1. Quantitative high-temperature combustion

Combustion elemental analysers perform a “flash combustion” of solid samples to  $\text{CO}_2$ ,  $\text{N}_2$ ,  $\text{SO}_2$ , and  $\text{H}_2\text{O}$ ; the reaction products are entrained in a stream of helium (approximately 100 mL/min  $\text{He}$ ), and either chemically trapped or separated on a GC column. The commercially available elemental analysers that are “continuous-flow-IRMS compatible” are all based on the original instrument from Carlo Erba, whose innovation was the use of the GC column to separate the reaction products. The analyte peaks enter the mass spectrometer via an open split interface (Fig. 3a) which performs a variety of tasks, including reduction of the flow rate down to 0.4 mL/min of  $\text{He}$ , dilution of large peaks to bring them within the dynamic range of the instrument, and insertion of pulses of reference gas into the  $\text{He}$  carrier stream [6].

A great deal of information can be gathered from a single combustion, including  $\delta\text{C}$ ,  $\delta\text{N}$ , atom%  $\text{C}$  and  $\text{N}$ , wt%  $\text{C}$  and  $\text{N}$ , and  $\text{C}/\text{N}$ . In general,  $\text{C}$  and  $\text{N}$  are analysed together, and  $\text{S}$  is analysed separately (Fig. 3b). The upper limit on  $\text{C}/\text{N}$  ratio that can be analysed is determined by the ability of the interface to perform dilutions on the larger peak (generally  $\text{C}$ ) without fractionation; with the ConFlo III split interface, the dynamic range has been significantly extended [7]. The lower limit of sample size is set partly by the blank and partly by the minimum required signal intensity. Systematic work on methods of blank reduction (e.g. zero blank auto-samplers) and blank correction [8] has led to ability to run sub-microgram samples of  $\text{C}$  and  $\text{N}$  (Fig. 3c, d).

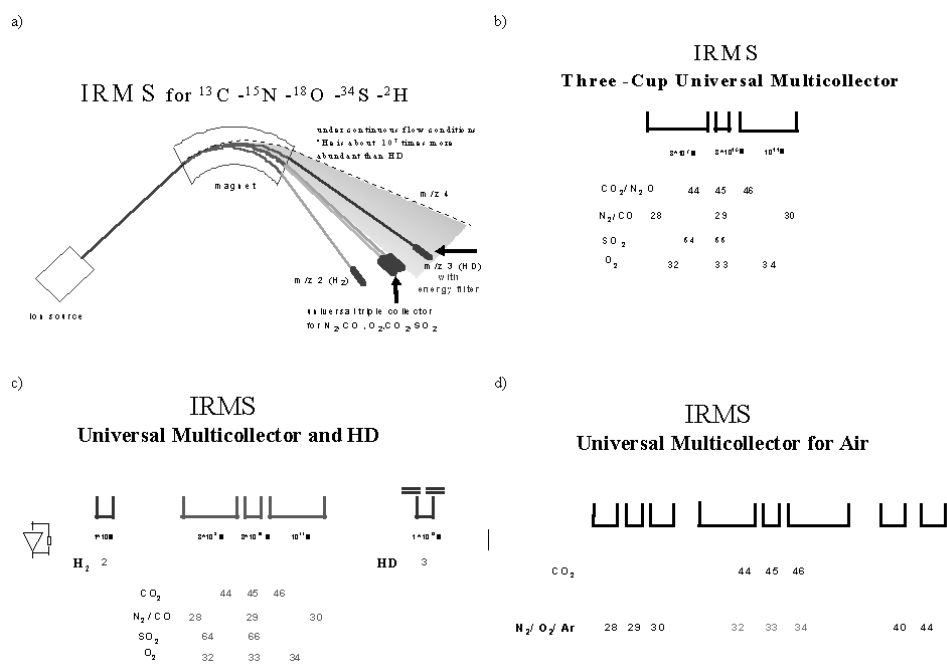


FIG. 2. Ion optical and Faraday collector configurations for IRMS. a) DELTA<sup>plus</sup> XL extended geometry for collection of  $\text{H}_2$  and CNOS gases on one image plane, and  $\text{HD}^+$  collector with built-in retardation lens to discriminate against the tail from the large  $4\text{He}^+$  peak, b) DELTA<sup>plus</sup> “universal triple collector” array, allowing collection of  $\text{N}_2$  and  $\text{CO}_2$  from a single elemental analyser combustion; c) DELTA<sup>plus</sup> XL collector array incorporating universal triple collector and  $\text{H}_2$  collectors; d) DELTA<sup>plus</sup> XL multicollector for simultaneous multicollection of major atmospheric gas species to allow measurement of  $\text{N}_2/\text{O}_2/\text{Ar}/\text{CO}_2$ .

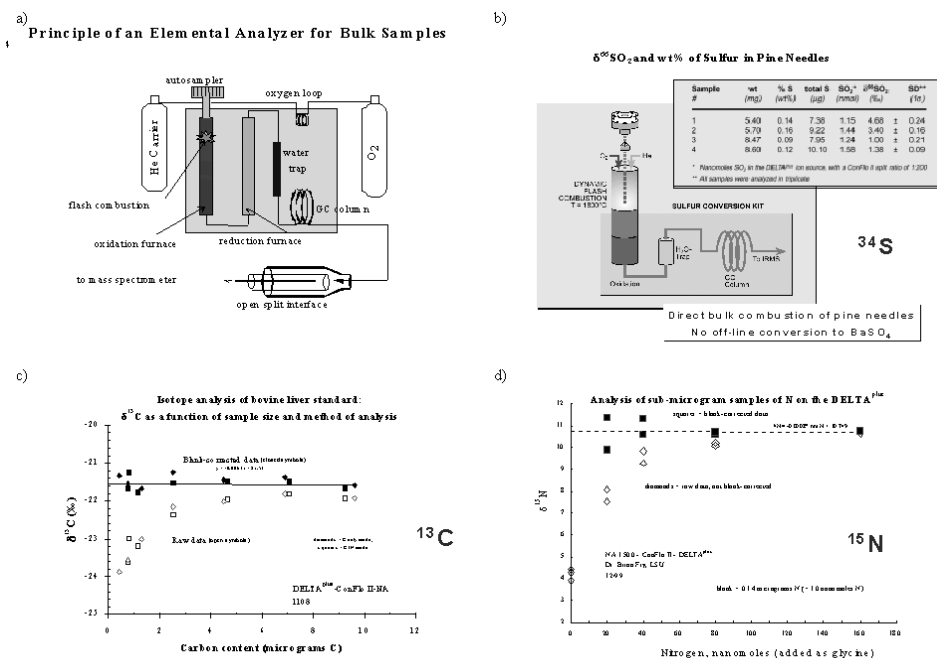


FIG. 3. Overview of combustion EA-IRMS. a) Schematic of combustion elemental analyser and split interface to IRMS; b) Analysis of S in organic material with a combustion EA; c) Analysis of microgram quantities of carbon requires blank correction; d) Analysis of sub-microgram quantities of nitrogen requires blank correction.

TABLE I. APPLICATIONS OF THE FINNIGAN MAT TC/EA (BSIA) CARBON REDUCTION ELEMENTAL ANALYZER

D/H and $^{18}\text{O}/^{16}\text{O}$ of $\text{H}_2\text{O}$	D/H of hydrous phases
Rain water	Water of hydration, e.g. gypsum
Saline water (ocean water, brines, bitterns)	Hydroxyl in silicates
Commercial mineral water	
Biological fluids (urine, saliva, blood)	
Biological fluids for doubly labeled water	
Plant water (leaf water, water of transpiration)	
Fluid inclusion water	
D/H and $^{18}\text{O}/^{16}\text{O}$ of foodstuffs	$^{18}\text{O}/^{16}\text{O}$ of minerals
Honey	Sulphates
Coffee beans	Phosphates
Vegetable oils	Nitrates ( $^{15}\text{N}/^{14}\text{N}$ also)
Dairy products (butter, cheese, milk)	Carbonates
Fruit juice pulp	Silicates
	Cocaine, heroin
D/H and $^{18}\text{O}/^{16}\text{O}$ of organic molecules of commerce	D/H and $^{18}\text{O}/^{16}\text{O}$ of fossil fuels
Sugar	Petroleum
Alcohol	Coal
Vanillin	
Essential oils	
Samples that can be analysed for time series	
D/H and $^{18}\text{O}/^{16}\text{O}$ of biopolymers	D/H and $^{18}\text{O}/^{16}\text{O}$ of biological tissues
Cellulose	Hair
Lignin	Fingernails
Silk	Hooves
Starch	Horns
Collagen	Feathers
Scales	

## 2.2. Quantitative high-temperature conversion

Although the combustion elemental analyser provided a practical solution to isotopic analysis of reduced C and N in a wide variety of solid materials, the isotopic analysis of oxygen and hydrogen proved to be a more difficult challenge, one that occupied many groups over a 30-year period, most notably for the analysis of  $^{18}\text{O}$  and D in cellulose [9]. The keys to successful analysis were the use of glassy C [10] at elevated temperatures ( $>1,450^\circ\text{C}$ ) and (for  $^{18}\text{O}$ ) the analysis of the CO rather than  $\text{CO}_2$ , all of which are incorporated into the Finnigan MAT TC/EA, a high-temperature C-reduction elemental analyser. Analogous to the analysis of  $\text{CO}_2$  and  $\text{N}_2$  from a single combustion on the combustion EA, it is possible with the TC/EA to analyse both CO and  $\text{H}_2$  from a single C reduction. The key to accurate analysis is quantitative conversion from substrate to analytical species; quantitative conversion of cellulose-H to  $\text{H}_2$ , and the linear response of the mass spectrometer for D/H is shown in Fig. 5a. One of the more novel applications of the TC/EA has been analysis of hair and fingernails for both O and H; Fig. 5b shows that large and correlated variation in  $\delta\text{D}$  and  $\delta\text{O}$  can be found in a single elephant hair that grew over a 1-year period. The TC/EA allows isotopic analysis of a wide variety of materials that were previously quite difficult (Table I). Sample-size requirements for the TC/EA are shown in Fig. 4d.

### 3. VOLATILE ORGANIC MOLECULES

The earliest publication that proposed a systematic approach to measuring the isotope ratios of GC peaks was from Japan in 1976 [11], in which a capillary GC was mated with magnetic sector single collector, and was used for metabolic purposes. In the United States, the Hayes group introduced “on-line combustion” to convert the GC peaks to CO<sub>2</sub> and N<sub>2</sub> prior to analysis on a magnetic sector single collection, which method was described as “isotope ratio monitoring-GCMS” [12, 13]. In 1984 in France, a capillary GC was mated with an isotope ratio mass spectrometer via a combustion oven, in order to make <sup>13</sup>C measurements of essential oils [14]. While the fundamental similarities of GC-IRMS (compound specific isotope analysis, CSIA) and EA-IRMS (bulk sample isotope analysis, BSIA) are several (Fig. 4a, b), development of the respective techniques were quite independent. The technique of GC-IRMS, which was originally focused on measurement of <sup>13</sup>C, was extended to include <sup>15</sup>N [15], <sup>18</sup>O [16], and, most recently, D [17].

The measurements of <sup>13</sup>C and <sup>15</sup>N in GC-resolved organic molecules is routinely made in >400 laboratories for a wide variety of applications (Fig. 6b, d). The measurement of D and <sup>18</sup>O content of organic molecules is significantly more difficult and is only now becoming generally accessible [18, 19]. The GC peaks are carried through a new high-temperature reactor that quantitatively converts the –H and –O of the organic molecules into peaks of CO and H<sub>2</sub> by pyrolysis; it has been shown that >1,400°C is required for quantitative conversion of –H into H<sub>2</sub> and –O into CO, because, at lower temperatures, thermodynamics allow the formation of such species as CH<sub>4</sub> and CO<sub>2</sub> [20]. The accurate and precise measurement of D/H from H<sub>2</sub> peaks in a He matrix presents a number of analytical challenges largely related to the tailing from the abundant <sup>4</sup>He<sup>+</sup> onto the minor HD<sup>+</sup> peak at m/z 3 (Fig. 2a), as well as ion-molecule reactions that occur in the ion source that produce H<sub>3</sub><sup>+</sup>. These difficulties have been identified and overcome [21], and it is now possible to accurately and precisely measure the D and <sup>18</sup>O contents of nanomolar amounts of individual organic compounds (Fig. 4c).

Initial survey work indicates that variations in D in organic molecules are large (up to 300‰ in n-alkanes) and that the isotopic composition of biomarkers has significant potential as a proxy for paleoclimate [18]. The more isotopes that can be brought to bear on a problem, the better are the chances of an unambiguous solution. The ability to measure multiple isotope ratios in a single molecule allows “stable isotope fingerprinting” of a wide variety of molecules. Three isotope ratios from a single compound can be measured in a variety of commercially important molecules, including ethanol and flavour compounds (e.g. benzaldehyde, vanillin, linalool) with the application being to determine their origin in an effort to detect adulteration or substitution [22].

The measurement of <sup>13</sup>C, <sup>18</sup>O, and D in the ethanol molecule is under intensive investigation (Fig. 6c), because IRMS provides an attractive alternative to existing NMR techniques. The ethanol-bearing sample is sampled by a GC autosampler using headspace-sampling techniques, and the capillary GC resolves ethanol from water and congeners. The purified ethanol is either combusted on-line for analysis for <sup>13</sup>C (C<sub>2</sub>H<sub>6</sub>O + 3O<sub>2</sub> = 2CO<sub>2</sub> + 3H<sub>2</sub>O) or quantitatively decomposed by pyrolysis at elevated temperatures (>1,400°C) on-line for analysis of <sup>18</sup>O and D (C<sub>2</sub>H<sub>6</sub>O = CO + 3H<sub>2</sub> + C).

### 4. ATMOSPHERIC GASES

While analysis of atmospheric gases presents analytical challenges that are quite distinct from those that had to be solved to use EA and GCs, the incorporation of continuous-flow methodologies has led to very significant advances in the isotopic composition of individual gas species. The problem with trace gases is, of course, their low concentration (Fig. 7d), which has hindered their direct measurement.

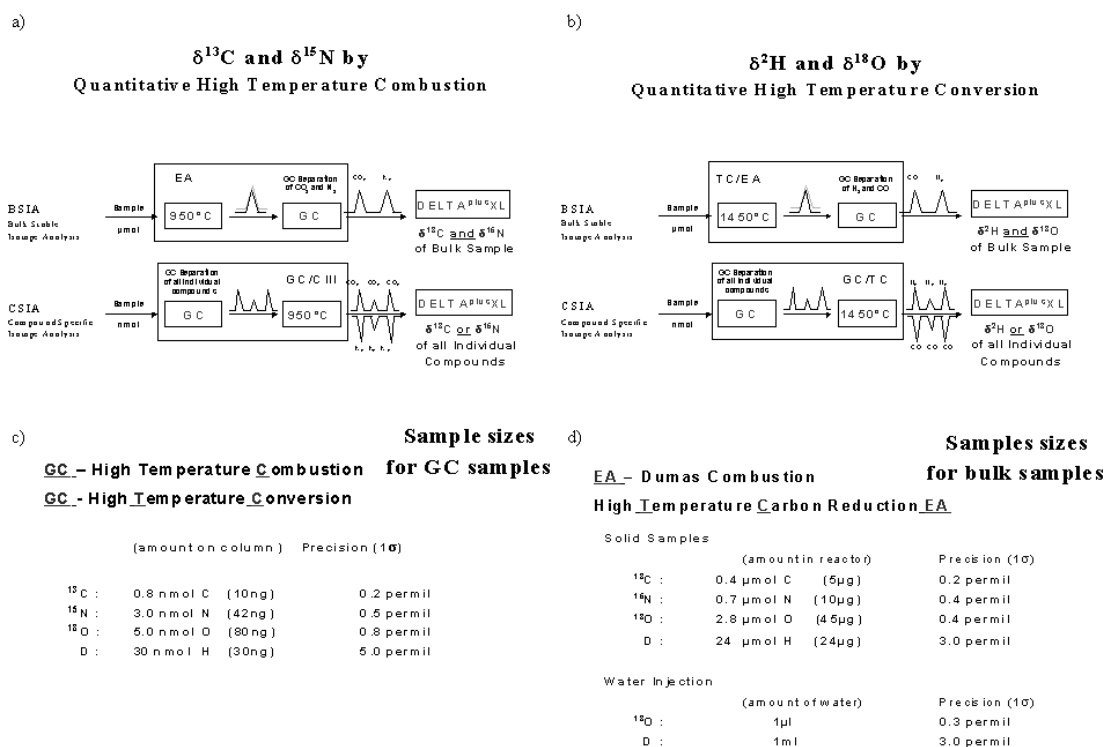
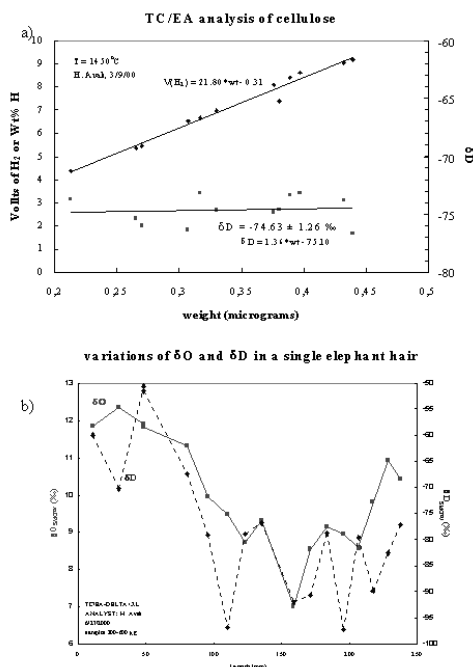


FIG. 4. Schematics and analytical figures of merit for EA-and GC-IRMS. a) Comparative schematics of GC-and elemental analyzer (EA) combustion for analysis of  $^{13}\text{C}$  and  $^{15}\text{N}$ ; b) Comparative schematics of GC/TC- and elemental analyzer (TC/EA) “pyrolysis” for  $^{18}\text{O}$  and D; c) Sample size requirements and analytical precision for  $^{13}\text{C}$  and  $^{15}\text{N}$  (GC/C) and  $^{18}\text{O}$  and D (GC/TC); d) Sample size requirements and analytical precision for  $^{13}\text{C}$  and  $^{15}\text{N}$  (EA) and  $^{18}\text{O}$  and D (TC/EA), and sample sizes for high-temperature C reduction of  $\text{H}_2\text{O}$  with the TC/EA.







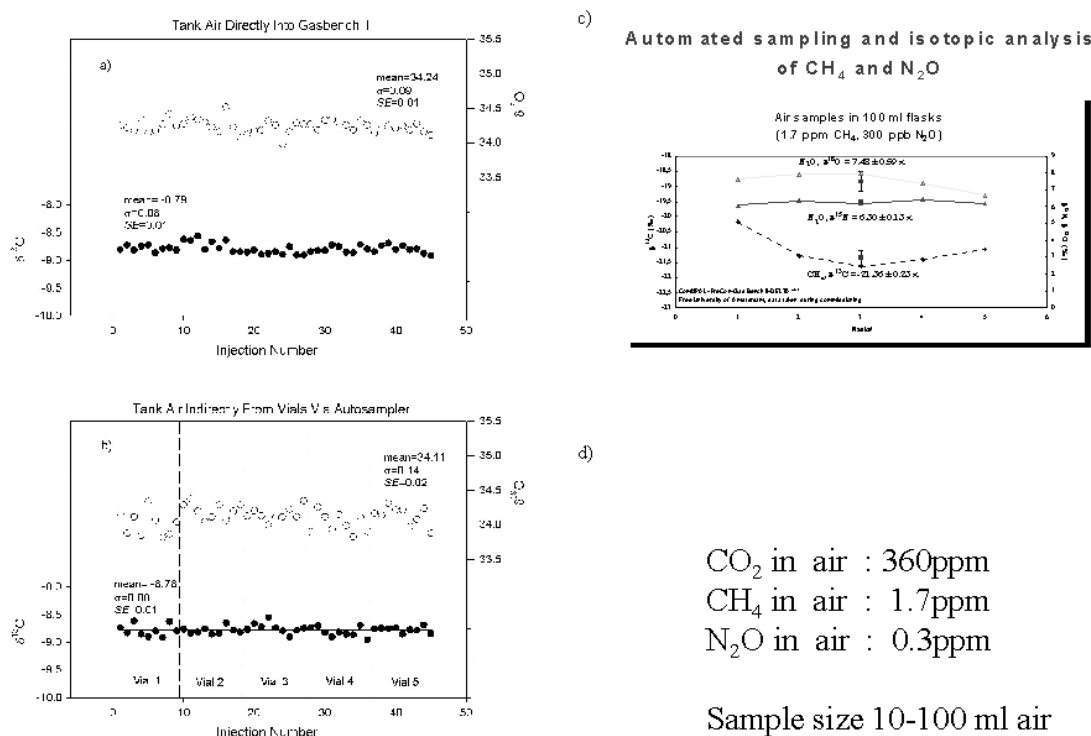


FIG. 7. Isotopic analysis of atmospheric trace gases; a) Sequential measurements of carbon and oxygen isotope ratios of  $CO_2$  in air introduced directly into the GasBench II by bypassing the autosampler; b) introduced from 10-mL septum capped vials via the autosampler; c) Automated sampling and analysis of both  $CH_4$  and  $N_2O$  in air, d) Natural abundances of  $CO_2$ ,  $CH_4$  and  $N_2O$  in air.

There is now a wide selection of methods for isotopic analysis of water, and the analyst can choose the most appropriate methodology. Water can be analysed using both  $CO_2$  and  $H_2$  equilibrations and headspace-sampling techniques. Carbon reduction at elevated temperature with TC/EA can be used to analyse both O and H on single sub-microliter samples of water ( $H_2O + C = H_2 + CO$ ) [28]. Similarly, but limited to analysis of H only, water can be reduced to  $H_2$  on chromium in a continuous-flow reactor in an elemental analyser. Nanoliter amounts of water can be analysed also by combining capillary GC transport with high-temperature pyrolysis reactors.

## 6. DISSOLVED COMPONENTS

The isotopic analysis of dissolved components (nitrate, sulphate, phosphate, DIC, DOC) has lagged behind that of all other components. The practices of continuous flow developed for EA-IRMS and GC-IRMS are now being applied to these problems and progress has been rapid. Microgram quantities of nitrate, sulphate and phosphate can all be analysed for  $^{18}O$  by high-temperature C reduction on the TC/EA, and  $^{34}S$  and  $^{15}N$  on the EA.

## 7. SUMMARY

Oxidation of organic C, N and organic and inorganic S by combustion to  $CO_2$ ,  $N_2$ , and  $SO_2$  is a relatively mature technique with a worldwide installed base of >600 EA and >400 GC-combustion systems. The use of pyrolysis and C-reduction techniques for quantitative conversion of organic and inorganic O and H to  $CO$  and  $H_2$  for both bulk and compound specific analysis is emerging rapidly, but is still in its infancy. The installed base is about sixty-five each of GC-pyrolysis and TC/EA systems, and the number is growing rapidly. Sample sizes for O measurements are in the same range as those in combustion mode but, because of the lower abundance of D and lower ionization efficiency

of H<sub>2</sub>, about ten times more sample is required for D/H measurements. Quantitative carbon reduction of organic and inorganic O allows measurements hitherto difficult or impossible, and thus offers considerable room for exploitation. Headspace sampling, followed by GC separation and cryofocusing offers considerable potential for the development of new methods of sample preparation in the future. All forms of CF-IRMS can be used to analyse water, with dramatic improvements in throughput and reduction in sample size, but none in precision or accuracy. The analysis of aqueous species is now being affected by CF-IRMS techniques.

## REFERENCES

- [1] BARSDALE, R. J., DUGDALE R.C., Rapid conversion of organic nitrogen to N<sub>2</sub> for mass spectrometry: an automated Dumas procedure, *Anal. Biochem.* **13** (1965) 1–5.
- [2] OTSUKI, A., et al., Simultaneous measurements and determinations of stable carbon and nitrogen isotope ratios, and organic carbon and nitrogen contents in biological samples by coupling of a small quadrupole mass spectrometer and modified carbon-nitrogen elemental analyser, *Intl. J. Mass Spectrom. Ion Phys.* **48** (1983) 343–346.
- [3] PRESTON, T., OWENS, N.J.P., Interfacing an automatic elemental analyser with an isotope ratio mass spectrometer: The potential for fully automated total nitrogen and nitrogen-15 analysis, *Analyst* **108** (1983) 971–977.
- [4] PICHLMAYER, F., BLOCHBERGER, K., Isotopenhäufigkeitsanalyse von Kohlenstoff, Stickstoff und Schwefel mittels Gerätekopplung Elementaranalysator-Massenspektrometer, *Fresenius Z. Anal. Chem.* **331** (1988) 196–201.
- [5] HILKERT, A.W., et al., “New IRMS technologies—high temperature conversion for bulk analysis of organic and inorganic samples”, *Proc. ASI 99*, Göttingen, Germany (1999).
- [6] ANOYMOUS, ConFlo II, Elemental Analyzer-IRMS Interface, Finnigan MAT GmbH, Bremen (1996).
- [7] WERNER, R.W., et al., ConFlo III—An interface for high precision  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  analysis with an extended dynamic range, *Rapid Comm. Mass Spectrom.* **13** (1999) 1237.
- [8] AVAK, H., FRY, B., EA-IRM: Precise and Accurate Measurement of  $\delta^{15}\text{N}$  on <10  $\mu\text{g}$  N, Application Flash Report # G29, Finnigan MAT GmbH, Bremen (1999).
- [9] DOUTHITT, C.B., Pyrolysis, Calcining, Thermolysis, Thermochemolysis and Related Methods of Sample Decomposition in CF-IRMS: An Annotated Comprehensive Bibliography, available on request from [cbdouthitt@compuserve.com](mailto:cbdouthitt@compuserve.com) (2001).
- [10] KOZIET, J., Isotope ratio mass spectrometric method for the on-line determination of oxygen-18 in organic matter, *J. Mass Spectrom.* **32** (1997) 103–108.
- [11] SANO, M., et al., A new technique for the detection of metabolites labeled by the isotope  $^{13}\text{C}$  using mass fragmentography, *Biomed. Mass Spectr.* **3** (1976) 1–3.
- [12] MATTHEWS, D.E., HAYES, J.M., Isotope-ratio-monitoring gas chromatography-mass spectrometry, *Anal. Chem.* **50** (1978) 1465–1473.
- [13] MATTHEWS, D.E., HAYES, J.M., “Isotope-ratio-monitoring gas chromatography mass spectrometry”, *Stable Isotopes, Proc. Third Int. Conference* (KLEIN, E.R., KLEIN, P.D., Eds.), Academic Press, New York (1979) 95–100.
- [14] BARRIE, A., et al., Gas chromatography-stable isotope ratio analysis at natural abundance levels, *Biomed. Mass Spectrom.* **11** (1984) 583–588.
- [15] MERRITT, D.A., HAYES, J.M., Nitrogen isotopic analyses of individual amino acids by isotope-ratio-monitoring gas chromatography/mass spectrometry, *J. Amer. Soc. Mass Spectrom.* **5** (1994) 387–397.
- [16] BRAND, W., et al., Compound-specific isotope analysis: extending towards  $^{15}\text{N}/^{14}\text{N}$  and  $^{18}\text{O}/^{16}\text{O}$ , *Org. Geochem.* **21** (1994) 585–594.
- [17] TOBIAS, H.J., et al., High-precision D/H measurement from hydrogen gas and water by continuous-flow isotope ratio mass spectrometry, *Anal. Chem.* **67** (1995) 2486–2492.
- [18] WARD, J.A.M., et al., Hydrogen isotope fractionation during methanogenic degradation of toluene: potential for direct verification of bioremediation, *Environ. Sci. Technol.* **34** (2000) 4577–4581.

- [19] XIE, S., et al., Palaeoclimate records in compound-specific  $\delta D$  values of a lipid biomarker in ombrotrophic peat, *Organic Geochem.* **31** (2000) 1053–1057.
- [20] BURGOYNE, T., HAYES, J., Quantitative production of  $H_2$  by pyrolysis of gas chromatographic effluents, *Anal. Chem.* **70** (1998) 5316–5141.
- [21] HILKERT, A.H., et al., Isotope ratio monitoring GCMS of D/H by high temperature conversion isotope ratio mass spectrometry, *Rapid Comm. Mass Spectrom.* **13** (1999) 1226–1230.
- [22] HÖR, K., et al., “On-line HRGC-IRMS determination of  $\delta^{13}C$  and  $\delta^{18}O$  values of flavor compounds-on the way to multi-element isotope ratio analysis for authenticity assessment”, *Frontiers of Flavor Science* (SCHIEBERLE, P., ENGLE, K.H., Eds.) (2000). ISBN 3-00-005556-8.
- [23] BRAND, W.A., PreCon: A fully automated interface for the pre-GC concentration of trace gases in air for isotopic analysis, *Isotopes Environ. Health Stud.* **31** (1995) 277–284.
- [24] TU, K., et al., Using septum capped vials with continuous flow isotope ratio mass spectrometric analysis of atmospheric  $CO_2$  for Keeling plot applications, *Rapid Comm. Mass Spectrom.* (submitted).
- [25] RICE, A.L., et al., Continuous flow measurement of  $\delta^{13}C$  and  $\delta D$  in atmospheric  $CH_4$ , 2000 Fall Meeting, American Geophysical Union (2000).
- [26] HORITA, J., Hydrogen isotope analysis of natural waters using an  $H_2$ -water equilibration method: A special implication to brines, *Chem. Geol.* **72** (1988) 89–94.
- [27] GEHRE, M., Sample preparation device for quantitative hydrogen isotope analysis using chromium metal, *Anal. Chem.* **68** (1996) 4414–4417.
- [28] SHARP, Z.D., et al., A rapid method for the determination of hydrogen and oxygen isotope ratios from water and solid hydrous substances, *Chem. Geol.* (in press).

## CHARACTERIZATION AND DYNAMICS OF PARTICULATE ORGANIC MATTER IN A RESTORED RIVER FLOODPLAIN SYSTEM

F. ASPETSBERGER, F. HUBER, S. KARGL, B. SCHARINGER, P. PEDUZZI, T. HEIN

Institute of Ecology and Conservation Biology,  
University of Vienna, Vienna, Austria

### Abstract

Connections between rivers and their adjacent floodplains influence the quality and quantity of organic matter and productivity within the water column. Changing patterns lead to alternating sources of particulate organic matter (POM). In many temperate rivers, anthropogenic influence on riverine wetlands has been exerted by damming. The River Danube downstream of Vienna still represents a floodplain-system with relatively pristine hydrological-exchange patterns, where different floodplain segments exhibit gradients of connectivity according to river distance and inflow areas. Particulate organic matter dynamics are mainly controlled by the river and follow changes in autochthonous and allochthonous sources of POM. Elemental composition and stable-isotope signatures can be used as a tool to investigate compositional quality and POM. Mean  $\delta^{13}\text{C}$  signatures and C:N ratios of autochthonous POM were significantly lower than for riverine POM. Further proof for the applicability of this method was found in significant relationships between  $\delta^{13}\text{C}$  values and C:N ratios. Similar trends were found between C:N ratios and  $\delta^{15}\text{N}$  signatures, particulate organic carbon (POC):Chl-*a* and  $\delta^{15}\text{N}$ , and POC:Chl-*a* and C:N ratios. Based on these findings, conclusions about the biological relevance of POM are drawn and the importance of floodplains for ecosystem productivity is emphasized.

### 1. INTRODUCTION

Rivers are long known as critical links in global cycling of organic matter, usually accumulating and transferring material of various terrestrial origins to the ocean. The dynamics of organic matter within a lotic system are determined mainly by stream morphology and hydrology [1]. Particulate organic matter (POM), which is considered in this study, comprises up to 50% of the total organic carbon at lotic sites. It is defined operationally as all of the material that is retained on a membrane filter.

The major component of terrestrial POM is plant material, chiefly cell walls, consisting mostly of structural polysaccharides, which are of minor importance for microorganisms and detritivores. In aquatic systems, amounts of detritus are often substantially greater than those of living substance. In rivers, POM can consist almost completely of detritus, whereas in lakes the presence of algae results in a higher proportion of the living component. Usually the POM in rivers consists of leaf litter, algal debris, plankton, and eroded substrate. The changes to which the POM is subjected are often very short term and dramatic, e.g. the appearance and subsequent breakdown of algal blooms [2].

Several functional aspects need to be considered when investigating the dynamics of POM. On the one hand, POM can provide information about the sources of organic material in a river. It can either be of terrestrial origin (soil organic matter, terrestrial litter) from upstream inputs, or be produced by the biota of the water body (macrophytes, algae). On the other hand, POM is an important substrate for microorganisms. It increases surface area for microbial activity, and thereby enlarges the transformation capacity. As a consequence, POM is subjected to a continuum of degradation.

Floodplains are known to alter the structure and quantity of detritus [3] and to act as important storage and processing areas for organic matter [4], and they are of crucial importance for ecosystem productivity in tropical [5] and temperate river-floodplain systems [6]. In floodplain ponds, there is a distinct dynamic in changes of source of POM. The production of the floodplain water body itself is regarded as an autochthonous source, and allochthonous sources can be inputs from the main river or of terrestrial origin.

As a consequence, the hydrological connectivity with the river—which determines the extent of water exchange—is of major importance for the change between autochthonous and allochthonous POM sources. At low discharge rates other factors seem to be operating, such as the above-mentioned autochthonous production and terrestrial inputs from the surrounding inundation areas.

## 2. STUDY SITE

The Danube is one of the major rivers of Central Europe. On its Austrian stretch (ca. 350 km) morphological conditions alternate between canyons with narrow riparian zones and braided watercourses with alluvial floodplains. River control and damming have resulted in straightening, increasing flow velocity, and reduced exchange conditions between the river and its backwaters [7]. Over the past 50 years, more than 90% of the upper Danube and its major tributaries have been dammed for hydropower production. The remaining few stretches have been severely affected by control measures. The river reach downstream from Vienna to Bratislava has been strongly impacted, nevertheless it represents one of the last remnants of a river floodplain system. Therefore, the Alluvial Zone National Park was formed in 1996 to preserve these remnants and provide recommendations for restoration.

deduce general patterns of water exchange between the main river and its floodplains, and their consequences for POM dynamics. Because this system still exhibits relatively pristine functional structures, it was used in this study to determine the degree of connectivity of each floodplain segment is determined by the height of the inflow areas. Connected situations are characterized by a riverine water level higher than the height of the inflow areas, whereas during disconnected situations the riverine water level is lower.

## 3. MAIN OBJECTIVES

- Investigate the elemental and isotopic composition of POM in a heterogeneous floodplain system.
- Determine the influence of the hydrological regime of the main river on the composition of POM.
- Evaluate stable-isotope analysis as a tool for the characterization of sources and diagenetic state of POM in an ecosystem approach.

## 4. APPROACH

Elemental analysis [POC, particulate organic nitrogen (PON), stable isotopes  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ] was used to investigate the quality of POM.

Particulate organic matter was sampled in the Danube River and in several adjacent floodplains of various connectivity over a 3-month period in spring and summer 1999; some additional samples were taken during a low-water period in early summer 2000. Batch cultures of phytoplankton ( $<60\ \mu\text{m}$ ) and bacteria were made to obtain isotopic signatures of these compartments.

## 5. RESULTS AND DISCUSSION

The concentrations of suspended solids and POM followed the water level of the Danube River, with maximum concentrations appearing at flood events. This applied not only to the river but also to the floodplain, with the latter reacting less strongly (Fig. 1). A comparison of suspended solids and POM reveals changing compositions of the suspended material with changing hydrology. Organic material was found in much higher concentrations in the floodplain, especially at low water levels and in the isolated floodplain.

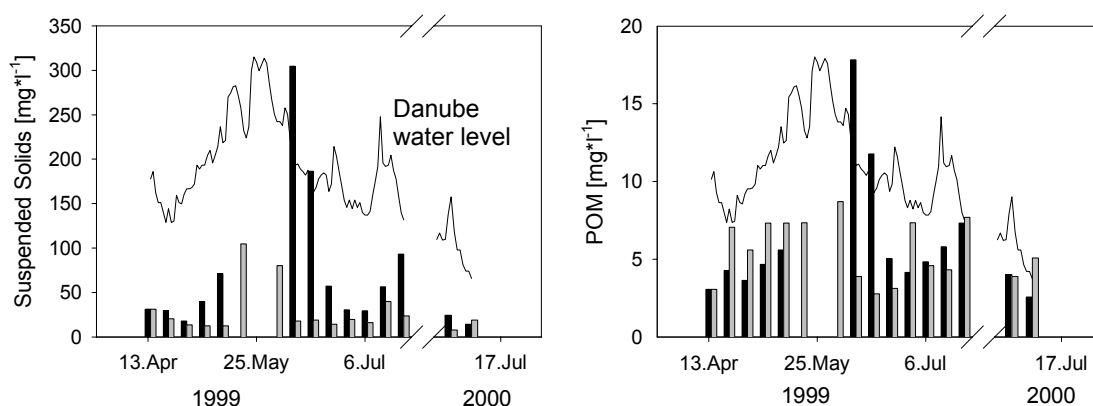


FIG. 1. Suspended solids and POM concentrations in the Danube River (black bars) and a disconnected floodplain (grey bars) during the investigation period. The line indicates the water level of the Danube River.

This points to changing sources of POM in such situations, uncoupled from inputs from the main river. Particulate organic carbon as well as PON concentrations in the floodplains rose conspicuously at high water levels, which points to an augmented input of diverse material with the influent riverine water.

Also, the C:N ratio showed maximum values at high water levels. Previous studies in the Danube floodplain system have shown that C:N ratios below 8 represent plankton-derived material, whereas values above 13 result from C of >90% detrital origin [8]. Values for vascular plants are much higher, usually commencing at C:N ratios of about 20. Our results—a positive correlation between water level and C:N ratio—illustrate that the relative proportion of non-living organic matter is enhanced by the Danube water.

Isotopic signatures also correlated significantly with the riverine water level. The  $\delta^{13}\text{C}$  signatures decreased with decreasing water levels, which points to a decreased terrestrial input of POM (Fig. 2). The lower signatures were close to values coming from aquatic production ( $<-25\text{‰}$ ), while those more enriched ( $>-20\text{‰}$ ) resembled signatures from detrital material. The  $\delta^{15}\text{N}$  signatures showed the opposite trend; they increased at low water levels (Fig. 2). This points to an ongoing enrichment of  $\delta^{15}\text{N}$  following the formation of more complex trophic structures in the floodplains.

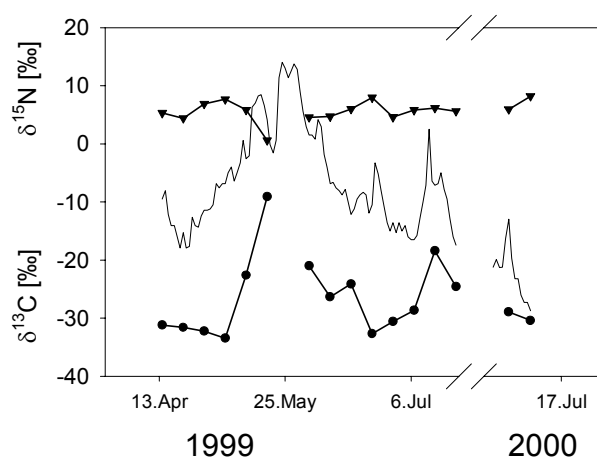


FIG. 2. Isotopic signatures of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  throughout the investigation period (line=Danube water level).

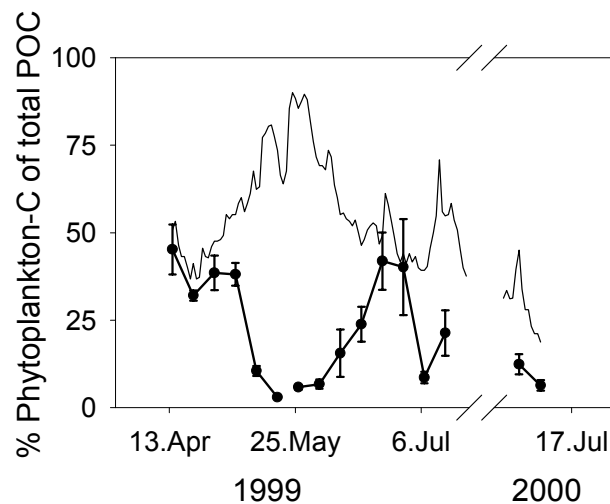


FIG. 3. Contribution of phytoplankton to the total POC pool during the investigation period (line = Danube water level).

Data from previous studies [8] allow comparisons of overall differences of isotopic signatures ( $\delta^{13}\text{C}$ ) among variously connected floodplain pools. Although all are linked to the water level of the main river, the different connectivity levels of various floodplains result in differences in  $\delta^{13}\text{C}$  signatures. The Danube River always showed the highest values, followed by the dynamically connected floodplain pools. Lowest  $\delta^{13}\text{C}$  signatures were always measured in the isolated floodplain. Only at the end of the investigation period when the river level was low, did these clear patterns show some variation.

Our data also enable us to look at the biotic compartments in the floodplain. Transforming chl-*a* concentrations and bacterial abundances into C-contents gives an indication of the relative contributions of these compartments to the POC pool. Matching the previously mentioned results, percentages of phytoplankton C and bacterial C of total POC peaked at low water levels and showed minima at high water (Fig. 3). Thus, the living fraction of POC increased in importance at low water levels, pointing to increased autochthonous production under such conditions.

Depending on the riverine water level, distinctions between connected and disconnected conditions for each floodplain pool were significant. Total suspended solids (TSS) revealed a clear difference between connected and disconnected conditions (Table I). This distinction was not found for total POC, which confirmed the time courses of these two parameters in the beginning: suspended solids were controlled by the regime of the river, whereas POC must have had other sources as well, probably autochthonous production and drainage basin effects.

The same was seen with the C:N ratio, which showed a significantly elevated proportion of C in connected situations. Greater nitrogen proportions, which are characteristic for a high biotic contribution to POM, were measured in disconnected conditions.

Differences in isotopic signatures brought more proof of autochthonous production of POM. Disconnected conditions resulted in  $\delta^{13}\text{C}$  signatures close to those of phytoplankton, whereas under connected conditions they are more enriched. Signatures of over  $-20\text{‰}$  are measured for predominantly detrital material ( $>90\%$ ) in this system. The  $\delta^{15}\text{N}$  signatures were higher under disconnected conditions, probably because of ongoing trophic enrichment with the heavier isotope.

Plotting the stable isotope signatures of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  against each other, again a distinct separation of connected and disconnected conditions can be seen (Fig. 4).

TABLE I. DIFFERENCES IN ELEMENTAL PARAMETERS BETWEEN CONNECTED AND DISCONNECTED CONDITIONS, CALCULATED ACCORDING TO THE HEIGHT OF THE INFLOW AREA AND THE WATER LEVEL OF THE DANUBE RIVER ON THE DAY OF SAMPLING

Parameter	Disconnected	Connected	Significance
TSS (mg L <sup>-1</sup> )	21.5 ± 1.9 <sup>a</sup>	52.2 ± 8.6	**
POC (mg L <sup>-1</sup> )	4.31 ± 0.4	5.21 ± 0.5	—
C:N ratio	6.76 ± 0.4	10.4 ± 1.0	***
δ <sup>13</sup> C (‰)	-28.3 ± 1.0	-20.1 ± 1.5	***
δ <sup>15</sup> N (‰)	6.45 ± 0.3	4.86 ± 0.3	***

<sup>a</sup>Standard error.

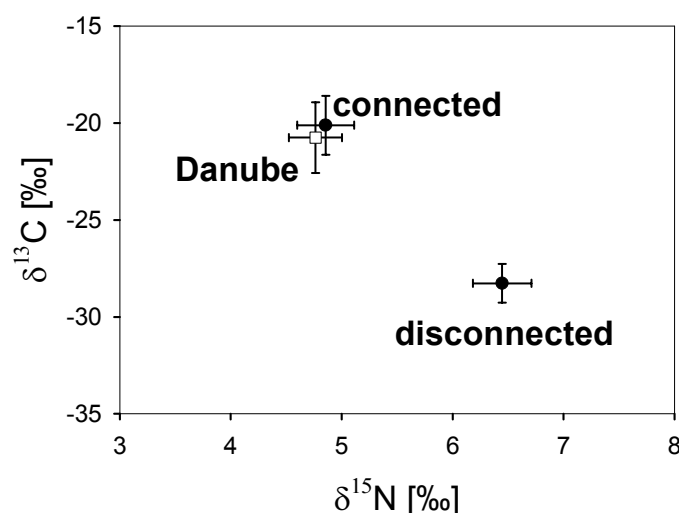


FIG.4. Differences in isotopic signatures between connected and disconnected conditions, calculated according to the height of the inflow area and the water level of the Danube River on the day of sampling.

Usually, such a graph is used to reconstruct trophic relationships in an ecosystem. In this case, one can deduce that connected and disconnected conditions result in distinct isotopic patterns.

Connected situations were dominated by allochthonous dynamics with values very close to those of the Danube River, showing the massive riverine water input. In contrast, disconnected situations were of a rather autochthonous character with isotopic signatures resembling those reported for phytoplankton.

As a conclusion from these findings, it can be deduced that even though the concentration of total POM seemed not to be directly related to the riverine water regime, its composition was markedly affected, showing significant differences under contrasting hydrological conditions.

Finally, the elemental parameters plotted against each other also showed the clear difference between allochthonously and autochthonously dominated conditions. These correlations support the use and validity of elemental analysis to characterize changing dominances in a river floodplain system. One example is presented here: increased δ<sup>13</sup>C signals correlated with increasing C:N ratios (Fig. 5), consistent with data from previous studies. Signatures similar to those of algae were measured at elevated proportions of N at C:N ratios usually found in algal material [9].



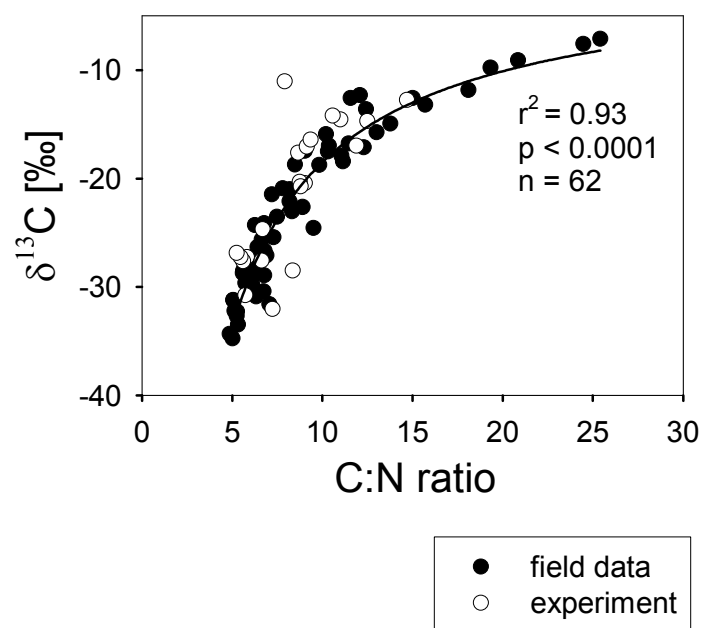


FIG. 5. Correlation of  $\delta^{13}\text{C}$  signal and C:N ratio, comparing field data with data obtained in phytoplankton batch cultures.

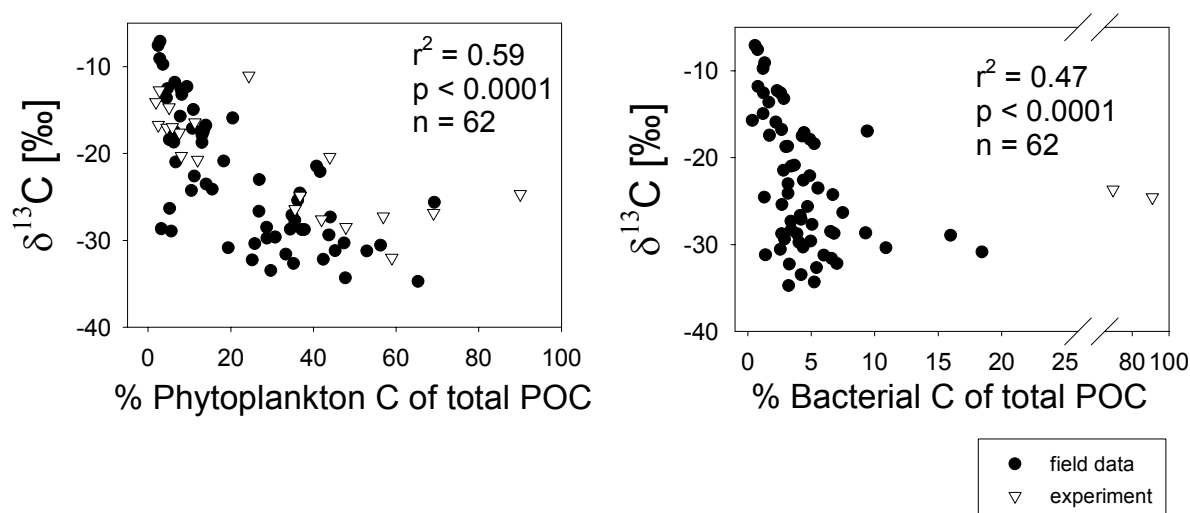


FIG. 6: Correlation of  $\delta^{13}\text{C}$  signals and biotic C-contribution, comparing field data with data obtained in phytoplankton batch cultures.

The data obtained in our cultures correlated with the field data (Fig. 6) confirming that bulk  $\delta^{13}\text{C}$  signatures as well as bulk C:N ratios were dominated by the phytoplankton contribution to POM. Furthermore, in situations of low  $\delta^{13}\text{C}$  and C:N ratios, high chlorophyll-*a* concentrations were found. There was a negative correlation with  $\delta^{15}\text{N}$ , supporting the previous findings.

The contribution of phytoplankton and bacterial carbon to the total POC pool matched the other results. Values ranged from 2.5% to 70% for phytoplankton and from 0.5% to 18% for bacterial C. For both compartments, rising percentages of POC resulted in depleted  $\delta^{13}\text{C}$  signatures (Fig. 6). Again it can be seen that phytoplankton dominated the total POC signal. For bacteria, a rather constant  $\delta^{13}\text{C}$  signature was measured, which obviously did not affect the signature of total POC to a significant degree, mainly attributed to the low percentages of bacterial C.

## 6. CONCLUSIONS

- Disconnected conditions are autochthonously dominated,  $\delta^{13}\text{C}$  signatures and C:N ratios are low whereas  $\delta^{15}\text{N}$  signals are enriched.
- Connected conditions with high input of allochthonous POM result in increasing proportions of the non-living component with enriched  $\delta^{13}\text{C}$  signatures and elevated C:N ratios.
- Hydrological control makes  $\delta^{13}\text{C}$  signatures a useful tool to assess the sources and the long term diagenetic state of POM in river floodplain systems
- $\delta^{15}\text{N}$  signatures are subjected to a biotic control, thus they contain information about the trophic complexity in a floodplain system on a shorter time scale.

Isotopic analysis of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures allows an ecosystem approach and establishes a complex picture of ecosystem dynamics by investigating only a few elemental parameters.

## 7. ECOSYSTEM IMPLICATIONS

The system investigated is a restored river floodplain, the study intended—in addition to other objectives—to provide information on the success of restoration efforts to bring the system back to a natural state. It was shown that floodplains are subjected to a very dynamic regime of riverine water levels that controls biotic and abiotic processes.

Intensified spiralling of suspended matter makes river floodplains “hot spots” of microbial activity, where high production rates are reached in short distances and spans of time.

It is noteworthy that the dynamics of disconnected floodplains cannot be compared to those of isolated waters, which act as sinks for C, mainly due to high stocks of aquatic macrophytes. In fact, the dynamic changes of connected and disconnected conditions occurring in the floodplains of this study area are of crucial importance to enabling the whole ecosystem to profit from the floodplain productivity.

Regarding the important role of POM in the turnover of organic matter—both as a degradation product and as a substrate for microorganisms—it can be stated that maintaining natural patterns of hydrological exchange between a river and its floodplain, either by protection or by restoration measurements, is essential for ecosystem function.

## REFERENCES

- [1] PETTS, G.E., AMOROS, C., *Fluvial Hydrosystems*, Chapman and Hall, London (1996).
- [2] ALLAN, D.J., *Stream Ecology*, Chapman and Hall, London (1995).
- [3] CUFFNEY, T.F., Input, movement and exchange of organic matter within a subtropical coastal blackwater river-floodplain system, *Freshwater Biol.* **19** (1988) 305–320.
- [4] ADMIRAAL, W., VAN ZANTEN, B., Impact of biological activity on detritus transported in the lower river Rhine: an exercise in ecosystem analysis, *Freshwater Biol.* **20** (1988) 215–225.
- [5] JUNK, W.J., *The Central Amazon Floodplain*, Springer, Berlin (1997).
- [6] TOCKNER, K., et al., Hydrological connectivity, and the exchange of organic matter and nutrients in a dynamic river-floodplain system, *Freshwater Biol.* **41** (1999) 521–535.
- [7] HEILER, G., et al., Hydrological connectivity and flood pulses as the central aspects for the integrity of a river-floodplain system, *Regul. Rivers Res. Manag.* **11** (1995) 351–361.
- [8] HEIN, T., et al., Hydrological connectivity in a river floodplain system: effects on nutrient and particulate organic matter dynamics and plankton development, Ph.D. Thesis, Univ. of Vienna (1999).
- [9] COFFIN, R.B., et al., Carbon isotopic compositions of estuarine bacteria, *Limnol. Oceanogr.* **34** (1989) 1305–1310.

## APPLICATION OF A SIMPLE CLEANUP TECHNIQUE FOR O AND N ISOTOPE ANALYSIS OF NITRATE IN NATURAL WATER SAMPLES

G. HABERHAUER, K. BLOCHBERGER, M.H. GERZABEK

Department for Environmental Research,  
Austrian Research Centre, Seibersdorf, Austria

### Abstract

The nitrogen isotopic ratio ( $\delta^{15}\text{N}$ ) has been used extensively as an indicator of the source of nitrate in the hydrosphere and as a measure of the degree of isotopic fractionation caused by chemical transformations such as denitrification. The analysis of O-isotopic composition of nitrate has many potential applications in studies of environmental processes. Oxygen-isotope nitrate analysis requires samples free of other oxygen-containing compounds. Non-nitrate-oxygen compounds will bias O-isotopic data. Therefore, an efficient clean-up method was developed to isolate nitrate from natural water samples. In a multi-step clean-up procedure using adsorption onto water-insoluble polyvinylpyrrolidone, removal of almost all other oxygen-containing compounds, such as fulvic acids, and isolation of nitrate, was achieved. The method was applied to set of surface- and soil-water samples. Samples free of non-nitrate oxygen were obtained. Subsequent combustion to  $\text{CO}_2$  was conducted and the  $^{18}\text{O}/^{16}\text{O}$  ratio values were determined.

### 1. INTRODUCTION

The global N cycle is increasingly influenced by industrial processes. Today, more atmospheric  $\text{N}_2$  is converted into biologically reactive forms by anthropogenic activities than by natural processes [1]. Deposition of N compounds may affect the N dynamics in forest ecosystems, which are characterized by efficient recycling of nutrients. The N-isotopic ratio ( $\delta^{15}\text{N}$ ) has been used extensively as an indicator of the source of  $\text{NO}_3^-$  in the hydrosphere and as a measure of the degree of isotopic fractionation [2, 3] caused by chemical transformations such as denitrification [4, 5]. However, those applications sometimes can be limited by the lack of discrimination or by local variations in the sources, sinks, and isotopic fractionation factors.

The  $\delta^{18}\text{O}$  value of  $\text{NO}_3^-$  has the potential to resolve some of the ambiguities presented by  $\delta^{15}\text{N}$  data, because some sources of O in  $\text{NO}_3^-$  are isotope-distinctive and because fractionation of the O isotopes is proportional to that of the N isotopes during common transformations such as denitrification. Therefore, precise and automated methods were developed and established for the analysis of both  $\delta^{15}\text{N}$  and  $\delta^{18}\text{O}$  for  $\text{NO}_3^-$  in environmental samples [6, 7]. Methods reported for  $\delta^{18}\text{O}$  analysis include combustion of  $\text{KNO}_3$  to  $\text{CO}_2$  with  $\text{Hg}(\text{CN})_2$  at  $550^\circ\text{C}$  [8, 9] with graphite at  $900^\circ\text{C}$  or  $1,400^\circ\text{C}$  and with catalysed graphite ( $\text{C} + \text{Pd} + \text{Au}$ ) at  $520^\circ\text{C}$  [10, 11].  $\delta^{18}\text{O}$  values of the sample are determined by subsequent isotope analysis of the evolved  $\text{CO}_2$  [11].

However, when investigating  $\delta^{18}\text{O}$  values in natural salt samples, one important, but often neglected, problem still remains. This is clean up of  $\text{NO}_3^-$  from environmental water samples containing complex matrices with dissolved organic matter and other O-bearing salts in concentrations of the same order as that of  $\text{NO}_3^-$ . Fulvic acids, which are regularly found in groundwater and surface-water samples, contain a certain amount of C-bound O. The O of the dissolved organic matter will also be converted to  $\text{CO}_2$  by combustion, and thus can bias the results of the isotopic measurements. Therefore, dissolved organic matter must be quantitatively removed before further combustion of the sample to  $\text{CO}_2$  and  $\delta^{18}\text{O}$  isotopic measurements of  $\text{NO}_3^-$  can be undertaken. Therefore, an efficient clean-up strategy based on cation exchange, adsorption of interfering compounds onto polyvinylpyrrolidone, and precipitation of sulphate with  $\text{BaCl}_2$  was developed for the quantitative elimination of non- $\text{NO}_3^-$  Oxygen-containing substances [12]. Description of the use of this method in conjunction with isotope analysis and its application is presented. Precipitation, surface-water, and soil-water samples were collected in a forest stand in the Austrian Tyrol and in an agricultural area in lower Austria.

## 2. DESCRIPTION OF THE METHOD

### 2.1. Apparatus and reagents

Total element analysis [total nitrogen ( $N_t$ ), total oxygen ( $O_t$ ) and total carbon ( $C_t$ )] was conducted with a Carlo Erba EA 1108 (Milan, Italy) elemental analyser. A Dionex DX-120 Ion Chromatograph (Sunnyvale, CA, USA) was used for  $NO_3^-$  determination in aqueous samples. Water-insoluble polyvinylpyrrolidone (Polyclar AT) was purchased from Serva (New York). The polymer was washed twice with water before use and passed through a 45- $\mu$ m cellulose acetate filter in order to remove smaller fractions. This step was crucial to the success of the method; without washing, high amounts of C and O, originating from the polymer, were found in the sample after treatment. The remaining polymer was dried before use.

Activated C and Isolute SPE column C18 material were obtained from Merck (Darmstadt, Germany) and IST (International Sorbent Technology, Mid Glamorgan, UK), respectively. Strong acid cation-exchange resin (Amberlite IR 120, Fluka, Buchs, Switzerland) was conditioned by washing with water. Double-distilled water was used exclusively. Sampling of soil water was carried out with suction cups. The samples were frozen immediately after collection. Nitrate concentrations on different sampling occasions varied between 10 and 60 mg L<sup>-1</sup>. All samples were part of a project studying N-dynamics in a forest ecosystem [13].

The residue, after clean up, was weighed and subjected to total N, O, C determination. The recoveries (%) on  $NO_3^-$ -O were calculated by dividing the amount of total O after the cleanup by the amount of  $NO_3^-$ -O of the original sample which was determined by ion chromatography. Recoveries of  $NO_3^-$ -N were calculated similarly.

For method A, an aliquot of the residue was dissolved in water for the determination of the  $NO_3^-$  concentration by ion chromatography. All clean-up procedures (A–C) were applied to both natural aqueous samples and control samples. A standard solution of  $NO_3^-$  (50 mg L<sup>-1</sup>) was used as the control.

### 2.2. Clean-up procedure

The clean-up techniques investigated consist of three main steps: cation exchange, adsorption of organic compounds onto solid adsorbents, and precipitation of sulphates with BaCl<sub>2</sub> after pH adjustment (Fig. 1). Evaporation to dryness yielded the purified samples, which were analysed for total N and total O. These results were compared to the  $NO_3^-$ -N and  $NO_3^-$ -O content of the original samples. Polyvinylpyrrolidone, activated charcoal, and a C18 material were investigated as adsorbents for organic compounds in methods A, B, and C, respectively. The recovery of N in the control samples was 90 to 100% for methods A and C, and about 40% for B. Thus, in the case of method B more than 50% of the  $NO_3^-$  was adsorbed onto the activated C.

The application of methods A to C for samples with a complex matrix (forest soil-water samples) led to the following results: Method C (clean up with cation exchange, adsorption onto C18 and precipitation of sulphate with BaCl<sub>2</sub>) yielded a recovery of 55% of N and contamination with O. Up to 96% of the O detected in the extract, therefore, did not originate from  $NO_3^-$ .

The low recovery of N in comparison to the control samples may be due to matrix-induced  $NO_3^-$ -adsorption on the C18 material. Method B (cation exchange, adsorption onto activated C, precipitation of sulphate with BaCl<sub>2</sub>) gave similar results (recoveries: 46% of N and 147% of O).

By the introduction of polyvinylpyrrolidone, non- $NO_3^-$ -O could be removed almost quantitatively. Recoveries of N and O were >90%. Ion chromatographic determination of the  $NO_3^-$  concentrations in the treated samples proved that nearly 100% of the total N and total O originated from  $NO_3^-$ .

## Natural Water Sample

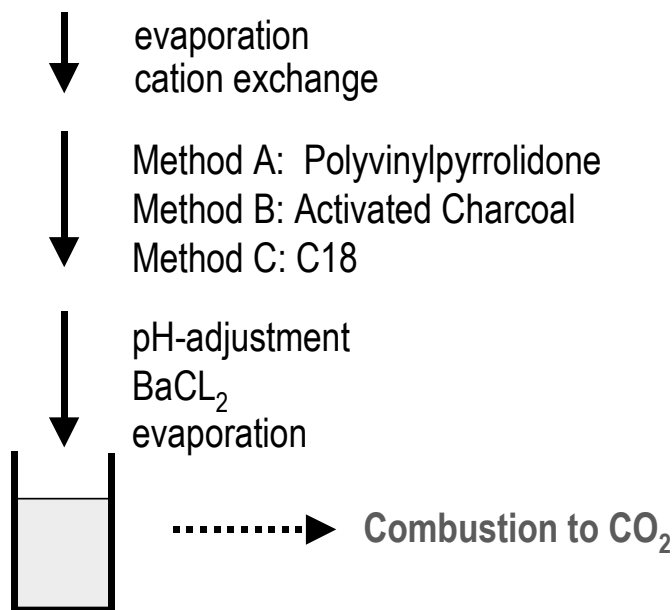


FIG. 1. Scheme of clean-up methods A, B and C.

### 2.3. Combustion to CO<sub>2</sub> and isotope determination

Only samples that were treated according to clean-up method A were subjected to combustion and  $\delta^{18}\text{O}$  stable-isotope analysis. For combustion, the  $\text{KNO}_3$  and  $\text{Hg}(\text{CN})_2$  (4/3 molar ratio) were weighed into quartz tubes, evacuated overnight, sealed and heated in an oven at  $560^\circ\text{C}$  for 6 h, and cooled slowly. Gases from combustion tubes were separated cryogenically;  $\text{CO}_2$  gas was transferred cryogenically into a sample tube for  $\delta^{18}\text{O}$  analysis. A Finnigan MAT 251 IRMS was used, the  $\delta^{18}\text{O}$  values were corrected according to [11], and were reported relative to VSMOW.

Measurements of isotopic abundances of  $\delta^{15}\text{N}$  were made with an IRMS (Finnigan MAT 251) coupled to an elemental analyser. The results were reported in the  $\delta$ -notation relative to air.

This method was applied to a set of soil, precipitation, and surface-water samples, which were collected either from a forest stand in the Austrian Tyrol and from surface, tap- and drain-water samples originating from lower Austria.

### 3. APPLICATION TO REAL SAMPLES

The isotopic composition of  $\text{NO}_3^-$  is used increasingly to determine sources and transformations of N in several ecosystems. Oxygen isotope ratios appear to be particularly useful, since they allow the differentiation between  $\text{NO}_3^-$  from deposition ( $\delta^{18}\text{O}_{\text{nitrate}}$  between +25 and +70‰),  $\text{NO}_3^-$  from fertilizer ( $\delta^{18}\text{O}_{\text{nitrate}}$  around +20‰) and  $\text{NO}_3^-$  derived from nitrification processes in soils ( $\delta^{18}\text{O}_{\text{nitrate}} < +15\text{‰}$ ) [7].

Before our method was applied to natural samples, we checked if any fractionation occurs due to the clean-up procedure. Artificial water samples containing a certain amount of pure  $\text{NO}_3^-$  were split into two aliquots. One aliquot was subjected to the clean-up procedure and then analysed for  $\delta^{18}\text{O}\text{-NO}_3^-$ , the other was analysed directly. The results showed that no significant difference between the  $\delta^{18}\text{O}$  of the  $\text{NO}_3^-$  after and before clean up (Fig. 2). Thus, we concluded that the method is a suitable procedure

for  $\delta^{18}\text{O}-\text{NO}_3^-$  analysis. The  $\delta^{18}\text{O}-\text{NO}_3^-$  values of samples of different origin are presented in Fig 2. The highest delta values were obtained with samples from precipitation, in close agreement with results from other studies [6,7].

In a set of samples obtained from an alpine site in the Tyrol [13], stable-isotope values were used to estimate the  $\text{NO}_3^-$  dynamics in the forest soil. The  $\delta^{15}\text{N}-\text{NO}_3^-$  values of the rainfall indicated that  $\text{NO}_3^-$  of different sources was deposited at that site. A significant correlation between the  $\delta^{15}\text{N}-\text{NO}_3^-$  values of the surface water and soil water were obtained, whereas no significant correlation between the  $\delta^{15}\text{N}-\text{NO}_3^-$  values of any precipitation sample with the surface water could be found [14]. The  $\delta^{18}\text{O}-\text{NO}_3^-$  values of some of these samples are plotted in Fig. 2. Significant differences between samples of precipitation origin and of surface/soil origin were obtained, which can be used to estimate the contribution of various pools of  $\text{NO}_3^-$  to natural water samples [6–8].

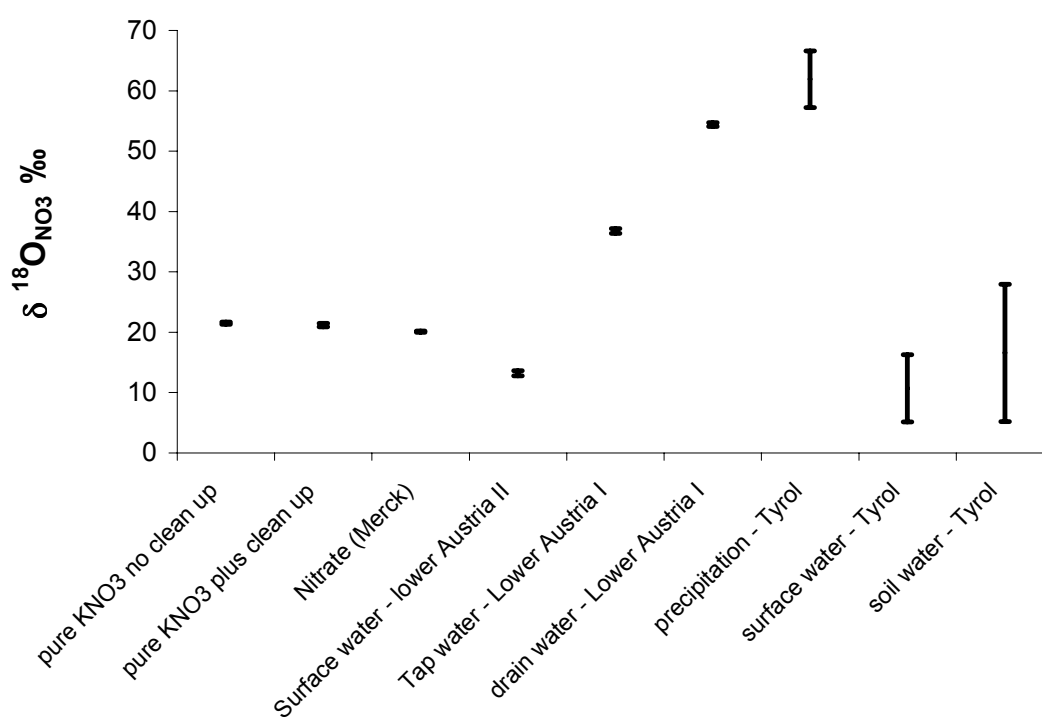


FIG. 2.  $\delta^{18}\text{O}_{\text{nitrate}}$  values of artificial and natural water samples. The Tyrol values represent a number of samples over a 2-year period (1998–1999).

#### 4. CONCLUSIONS

The determination of  $\delta^{18}\text{O}$  in natural water samples after combustion to  $\text{CO}_2$  requires the removal of all non- $\text{NO}_3^-$ -O. A new and efficient clean-up strategy based on cation exchange, adsorption of interfering compounds onto polyvinylpyrrolidone, and precipitation of sulphate with  $\text{BaCl}_2$  was developed for the quantitative elimination of non  $\text{NO}_3^-$ -O-containing substances. A comparison with other adsorbents such as activated C or C18 demonstrated that the application of water insoluble polyvinylpyrrolidone is crucial for the success of the clean-up procedure.

High recoveries and removal of almost all interfering O compounds were achieved using water-insoluble polyvinylpyrrolidone as adsorbent material, whereas other adsorbent materials, such as C18 or activated C, were found to be unsuitable. The results clearly demonstrate the importance of the development of matrix-specific clean-up strategies for sample preparation of O-isotope analysis. The

method presented allows simple High recoveries and  $\text{NO}_3^-$  isolation, without any fractionation, from precipitation, surface and soil water samples, were obtained from a forest site heavily loaded with other O-containing compounds.

## REFERENCES

- [1] NADELHOFFER, K.J., et al., Nitrogen deposition makes a minor contribution to carbon sequestration in temperate forests, *Nature* **398** (1999) 145–148.
- [2] LETOLLE, R., “Nitrogen-15 in the natural environment”, *Handbook of Environmental Isotope Geochemistry* Vol. 1 (FRITZ, P., FONTES J.C., Eds.), Elsevier, Amsterdam (1980) 407–433.
- [3] HÜBNER, H. “Isotope effects of nitrogen in the soil and biosphere,” *Handbook of Environmental Chemistry, The Terrestrial Environment*, Vol. 2 (FRITZ, P., FONTES, J.C., Eds.), Elsevier, New York (1986) 361–425.
- [4] WADA, E., UEDA, S. “Carbon, nitrogen and oxygen isotope ratios of  $\text{CH}_4$  and  $\text{N}_2\text{O}$  in soil ecosystems,” *Mass Spectrometry of Soils* (BOUTTON, T.W., YAMASAKI, S., Eds.) Marcel Dekker, New York (1996) 177–204.
- [5] EMMET, B.A., et al., Natural abundance of  $^{15}\text{N}$  in forests across a nitrogen deposition gradient, *Forest Ecol. Management* **101** (1998) 9–18.
- [6] WASSENAAR, L.I., Evaluation of the origin and fate of nitrate in the Abbotsford aquifer using the isotopes of  $^{15}\text{N}$  and  $^{18}\text{O}$  in  $\text{NO}_3$ , *Appl. Geochem.* **10** (1995) 391–405.
- [7] MAYER, B., BOLLWERK, S.M., Controls of Oxygen Isotope Ratios of Nitrate Formed During Nitrification in Soils, IAEA Report CSP-2, IAEA-SM-361, Vienna (2000).
- [8] VOERKELIUS, S., Isotopendiskriminierungen Bei Der Nitrifikation Und Denitrifikation: Grundlagen Und Anwendungen Der Herkunfts-Zuordnung Von Nitrat Und Stickstoffmonoxid, Dissertation, TU-München (1990).
- [9] DURKA, W., et al., Effects on forest decline on uptake and leaching of deposited nitrate determined from  $^{15}\text{N}$  and  $^{18}\text{O}$  measurements, *Nature* **372** (1994) 765–767.
- [10] LAUF, J.; GEBAUER, G., Online analysis of stable isotopes of nitrogen in  $\text{NH}_3$  NO and  $\text{NO}_2$  at natural abundance levels, *Anal. Chem.* **70** (1998) 2750–2756.
- [11] REVESZ, K., et al., Determination of  $\delta^{18}\text{O}$  and  $\delta^{15}\text{N}$  in nitrate, *Anal. Chem.* **69** (1997) 4375–4380.
- [12] HABERHAUER, G., BLOCHBERGER, K., A simple clean up method for the isolation of nitrate from natural water samples for O isotope analysis, *Anal. Chem.* **71** (1999) 3587–3590.
- [13] HERRMAN, F., et al., Description and evaluation of pollution impacts on forest ecosystems: results of long-term experiments, *Environ. Sci. Pollut. Res.* **1** (1998) 16–24.
- [14] HABERHAUER, G., et al., “Stabilisotopenanalytik von Nitrat zur Abschätzung der N-Dynamik im Waldboden und der N-Belastung des Bodenwassers am Mühleggerköpfl /Nordtiroler Kalkalpen,” Report 109 of the Forstliche Bundesversuchsanstalt, Forstliche Bundesversuchsanstalt, Vienna (2000) 99–109.

# CONTINUOUS FLOW PYROLYSIS TECHNIQUES FOR THE ISOTOPIC MEASUREMENTS OF OXYGEN-DEUTERIUM IN WATERS, ORGANIC AND INORGANIC COMPOUNDS

J. MORRISON, H. HERTLE

Micromass UK Ltd,

Wythenshawe, Manchester, United Kingdom

The technique of interfacing an elemental analyser (EA) with a stable isotope ratio mass spectrometer has, since its inception in 1983 [1], proven to be a remarkably versatile analytical tool for the measurement of carbon and nitrogen isotopes across a wide application base. By 1994, sulphur isotopes had been added to the list [2] and, more recently, those of oxygen and hydrogen. The analysis of oxygen and hydrogen involved a departure from the normal flash-combustion mode of operation to one of pyrolytic thermal decomposition of the sample [3–6].

This paper describes a new EA technique for the measurement of hydrogen isotopes in water, in a continuous-flow carrier stream of helium. The system is totally carbon free and is based on the use of chromium (patented) as the active reactor material. Water injected into this system is reduced, resulting in the quantitative release of hydrogen gas, which is then carried by the helium to the mass spectrometer and analysed for deuterium concentration. This technique exhibits remarkably low memory effects, excellent precision and accuracy, and addresses sample sizes down to 50 nL. In addition, it allows a very high sample throughput with individual sample analysis time of 3 min.

A EuroVector EA was configured with a chromium-packed reactor at a temperature of 1,050°C and fitted with a 1.5-m molecular sieve packed GC column. Samples were dispensed into 1.5-mL septa-sealed vials and placed on the carousel of an automated liquid autosampler device (EuroVector LAS2000). A sequence of three wash cycles was carried out on each sample prior to injection into the reactor through a heated septa-sealed injector port. A sample size of 0.5  $\mu$ L of water was chosen for the analysis. The liquid autosampler has the ability to measure up to 110 individual samples; each sample can be analysed up to 144 times. Hydrogen generated in the reactor is passed through the GC column and transported via an open split capillary into the source of a Micromass IsoPrime stable isotope ratio mass spectrometer.

A memory/accuracy/precision test was conducted on this system using a suite of standards including the primary standards SMOW and SLAP, secondary standard GISP, two laboratory standards from an external source (SSRW and INV1) and two in-house waters previously measured using this technique (HS and AS). Figure 1 shows the data in the order in which the standard groups were run. Table I shows the corrected values obtained for each group and the standard deviation for each standard.

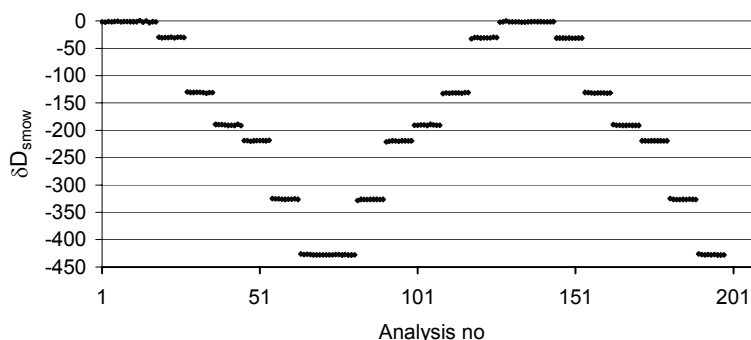


FIG. 1. Analysis number versus  $\delta D$  value for each sample.



TABLE I. STANDARDS DATA

Sample	Expected	$\delta D_{\text{SMOW}} \text{‰}$	$\Delta$ true-measured	Std. Dev.	n
SMOW	0	-0.3	0.3	0.8	18
HS	-29.8	-29.3	-0.5	0.4	9
SSRW	-131	-130.3	-0.7	0.6	9
GISP	-190	-189.9	-0.1	0.9	9
INV1	-219	-218.6	-0.4	0.5	9
AS	-326	-325.9	-0.1	0.4	9
SLAP	-428	-427.9	-0.1	0.5	18
AS	-326	-326.8	0.8	0.5	9
INV1	-219	-219.5	0.5	0.7	9
GISP	-190	-190.0	0.0	0.4	9
SSRW	-131	-131.2	0.2	0.4	9
HS	-29.8	-29.9	0.1	0.8	9
SMOW	0	-0.6	0.6	0.5	18
HS	-29.8	-30.5	0.7	0.2	9
SSRW	-131	-131.1	0.1	0.5	9
GISP	-190	-190.4	0.4	0.5	9
INV1	-219	-219.1	0.1	0.2	9
AS	-326	-326.4	0.4	0.6	9
SLAP	-428	-428.1	0.1	0.4	9
		Mean	0.14	0.53	

Here the measurements represent both natural abundance variability and a run order that minimizes memory effects. The smallest transition in delta is approximately 28‰ while the largest is 107‰. A total of 198 individual sample analyses are contained in Table I. There were no outliers in this data set. Total analysis time was 10 h, each analysis taking approximately 3 min.

This new technique has also been applied to the measurement of hydrogen isotopes in chlorinated hydrocarbons such as trichloroethane and trichloroethylene [4]. In addition, we have demonstrated  $^{18}\text{O}$  analyses of both organic and inorganic samples using a range of reactor configurations and temperatures.

## REFERENCES

- [1] PRESTON, T., OWENS, N.J.P., Interfacing an automatic elemental analyser with an isotope ratio mass spectrometer: the potential for full automated total nitrogen and nitrogen-15 analysis, *Analyst* **108** (1983) 971.
- [2] GIESEMANN, A., et al., On-line sulphur isotope determination using an elemental analyser coupled to a mass spectrometer, *Anal. Chem.* **66** (1994) 2816–2819.
- [3] FARQUHAR, G.D., et al., A rapid on-line technique for determination of oxygen isotope composition of  $\text{N}_2$ -containing organic matter and water, *Rapid Comm. Mass Spectrom.* **11** (1997) 1554–1560.
- [4] WERNER, R.A., et al., On-line determination of  $\delta^{18}\text{O}$  values of organic substances, *Anal. Chim. Acta.* **319** (1997) 159–164.
- [5] KOZIET, J., Isotope ratio mass spectrometric method for on-line determination of oxygen-18 in organic matter, *J. Mass Spectrom.* **32** (1997) 103–108.
- [6] KORNEXL, B.E., et al., On-line  $\delta^{18}\text{O}$  measurement of organic and inorganic substances, *Rapid Comm. Mass Spectrom.* **13** (1999) 1685–1693.

# DETERMINATION OF IODINE IN CEREAL GRAINS CULTIVATED IN AUSTRIA AND STANDARD REFERENCE MATERIALS BY NEUTRON ACTIVATION ANALYSIS

T. SHINONAGA, M.H. GERZABEK, K. MÜCK, J. CASTA  
Austrian Research Centre,  
Seibersdorf, Austria

## Abstract

Trace amounts of iodine in cereal grain samples cultivated at various locations in Austria were determined for the first time in this study, by radiochemical neutron activation analysis. For the dissolution of cereal grain samples and standard reference materials, two procedures, alkaline and acidic methods, were applied in the presence of an iodine carrier. A rapid, simple dissolution procedure with acidic solution was demonstrated. The analytical values in the cereal grains, as well as in standard reference materials, obtained by the different dissolution procedures were in good agreement within one standard deviation. The relative standard deviations for concentrations of  $0.1 \mu\text{g g}^{-1}$  of iodine were around 10%.

## 1. INTRODUCTION

Iodine is an essential nutrient for the metabolic function of thyroid hormones. Adequate iodine levels in food and feed plants are required in human and animal nutrition [1]. Furthermore, release of the long lived radionuclide  $^{129}\text{I}$  ( $T_{1/2} = 1.6 \times 10^7 \text{ yr}$ ) from nuclear-reactor accidents results in a small fraction of environmental iodine  $^{129}\text{I}$ . Iodine-129 is expected to behave in the environment similarly to stable iodine over a long time scale [2]. Therefore, in both contexts, the estimation of iodine concentrations in important foods is important. However, information on iodine contents in cereal grains is very limited, mainly due to the difficulty of determination of trace amounts. Neutron activation analysis has been frequently used as it is the most sensitive analytical technique. With very low levels, radiochemical separation techniques were additionally applied to instrumental neutron activation analysis. Isotope dilution mass spectrometry has also been used for the measurement of trace amounts of iodine since the 1980s [3]. Due to recent developments of instruments and analytical techniques, the inductively coupled plasma mass spectrometry (ICP-MS) is a means of simultaneously determining chlorine, bromine and iodine [4].

In this study, low concentrations of iodine in cereal grains cultivated in Austria and in four standard reference materials for plants and rock were determined by radiochemical neutron activation analysis (RNAA) and instrumental neutron activation analysis (INAA). Iodine in grain samples was determined for the first time in this study. The samples were dissolved in an alkaline or acidic solution and iodine was separated from the solution as palladium iodine. For the acidic dissolution, mixtures of three ratios of  $\text{HNO}_3$ ,  $\text{HCl}$ , and  $\text{HClO}_4$  were examined and compared. A simple, rapid dissolution procedure with acidic solution was demonstrated. The detection limits for each method and the standard deviations were estimated; precision and the accuracy are discussed.

## 2. EXPERIMENTAL

### 2.1. Sample preparation

#### 2.1.1. Standard preparation

Superior grade reagent KI was dissolved in distilled water containing a few drops of 6%  $\text{Na}_2\text{SO}_3$  and the solution was adjusted with  $\text{LiOH}$  to a pH of approximately 10. The solution ( $5 \mu\text{g I}$ ) was transferred to a filter paper and dried at room temperature. The filter paper was doubly sealed in a polyethylene bag for irradiation.

### *2.1.2. Austrian cereal grains and plant and rock standard reference materials*

The cereal grain samples analysed in this study were collected in various agricultural regions in Austria. They are classified as winter wheat, spring wheat, wheat, and winter rye.

Standard reference materials, wheat flour (SRM 1567a) [5], orchard leaves (SRM 1571) [6], and apple leaves (SRM 1515) [7] processed and distributed by the National Institute of Standards and Technology, and the rock-standard reference material (JF-1: mixture of orthoclases and albite) processed and distributed by the Geological Survey of Japan, were analysed.

## **2.2. Irradiation of samples and gamma ray measurements**

The cereal-grain samples were pulverized, and dried at 85°C for several days. The standard reference materials were similarly dried. About 1 g of plant sample and plant standard materials (except apple leaves) and about 0.5 g of rock sample were weighed and doubly sealed in polyethylene bags for irradiation. About 0.1 g of apple leaves were used for the RNAA and INAA.

Duplicate plant and rock samples were irradiated in a vial with a standard sample of iodine for 5 min in the boron carbide (B<sub>4</sub>C) irradiation facility (thermal neutron flux:  $5 \times 10^{12}$  n cm<sup>-2</sup> s<sup>-2</sup> epithermal/thermal neutron: ~30) of the ASTRA reactor at the Austrian Research Centre, Seibersdorf. In this cylindrical irradiation facility, the sample is shielded against thermal neutrons by a B<sub>4</sub>C layer surrounding the irradiation space. The polyethylene vial enclosing the samples and the standard was positioned between additional B<sub>4</sub>C holders during irradiation. Irradiation was also performed at the TRIGA reactor (thermal neutron flux:  $2.5 \times 10^{12}$  n cm<sup>-2</sup> s<sup>-2</sup> epithermal/thermal neutron: ~12) at the Atomic Institute of Austrian Universities, Vienna; the samples were irradiated for 20 to 25 min with this reactor.

Gamma rays of <sup>128</sup>I were measured by high-resolution gamma-spectroscopy on Ge(Li)-detectors at 442.9 keV for 15 to 25 min.

## **2.3. Determination of chemical yield**

Chemical yield was determined using <sup>125</sup>I measured with a low-energy photon counter (Li-intrinsic-detector) at 0.0355 keV for about 5 min. The determined values were corrected with the chemical yield.

## **2.4. Chemical separation procedure**

### *2.4.1. Alkaline dissolution*

The chemical separation of iodine by alkaline dissolution was performed mainly according to the procedure reported by Takagi et al. [8]. The irradiated sample was immediately transferred into a glass beaker and moistened with 1 mL of 0.2 M NaOH. A carrier solution of iodine (10 mg I) and the radioactive tracer <sup>125</sup>I were added and heated, gradually adding about 30 mL of sodium hypochlorite to dissolve the sample. After dissolution, the solution was made acidic by HCl and boiled to remove chlorine. The solution was passed through a glass filter to remove remnants, which were washed with distilled water. A few drops of 6% Na<sub>2</sub>SO<sub>3</sub> were added to this filtrate to reduce iodate ions to iodide ions. After 1 mg of Br<sup>-</sup> was added to the solution, PdCl<sub>2</sub> (15 mg as Pd) was administered and the iodine precipitated as PdI<sub>2</sub>. The precipitate was collected on a glass filter, fixed with tapes, and then measured by  $\gamma$ -spectroscopy.

### *2.4.2. Acidic dissolution*

Three different ratios of HNO<sub>3</sub>, HCl, and HClO<sub>4</sub> (1:3:3, 1:1:1, and 1:0:1) were examined for which is most suitable to dissolve the sample. The same procedure was applied for the chemical separation as described for alkaline dissolution, except for the pH adjustment.

### 3. RESULTS AND DISCUSSION

#### 3.1. Detection limit

The detection limits based on three standard deviations of background in the present study were about 0.4, 0.4, and 10 ng, and 0.7, 0.8, and 60 ng for the alkaline, and acidic dissolution, and non-destructive analysis, with the ASTRA and TRIGA reactors, respectively. With the ASTRA reactor, a lower detection limit was obtained than with the TRIGA, due to the higher neutron flux and the effective B<sub>4</sub>C shielding of the ASTRA facility. The ratio of epithermal/thermal neutron flux in the irradiation position is higher and thus the production of disturbing radionuclides, e.g. <sup>56</sup>Mn, was lower compared with <sup>128</sup>I.

#### 3.2. Acidic solution for sample dissolution

Three different ratios of acids were examined for the sample dissolution. A high yield of PdI<sub>2</sub> was obtained only from the HNO<sub>3</sub>:HCl:HClO<sub>4</sub> mixture at 1:3:3, with very little obtained from the other two solutions. Larsen and Ludwigsen reported on risk of iodine volatilization during sample dissolution by HNO<sub>3</sub> [9]. They dissolved biological samples with a mixture of HClO<sub>4</sub> and HNO<sub>3</sub>, in closed high-pressure steel bombs at 170°C to prevent loss of iodine. In this study, we decomposed the samples in a glass beaker at more than 200°C, since a rapid chemical separation was required. With the solution described above, the rapid and simple procedure for sample decomposition was available. Results obtained using the 1:3:3 mixture are shown in the Table I.

#### 3.3. Chemical yield

The chemical yields were typically 80 to 90%, and there were no significant differences between values obtained by alkaline and acidic dissolution procedures. Sample matrix had no significant influence on chemical yields, though those for the rock samples were about 100%.

#### 3.4. Accuracy of analysis for standard reference materials

The analytical results with the standard reference materials are shown in Table I. Three plant reference materials, wheat flour, orchard leaves, and apple leaves, and one volcanic rock standard reference material were analysed. The values of wheat flour, orchard leaves, apple leaves, and volcanic rock were 0.0016, 0.18, 0.30, and 0.0118 µg g<sup>-1</sup>, and their literature (non-certified) values were 0.0009, 0.17, 0.30, and 0.009 µg g<sup>-1</sup>, respectively. The relative standard deviations (RSD) of this study were 38, 17, 10, and 3.4%, for the wheat flour, orchard leaves, apple leaves, and volcanic rocks, respectively. The RSD for wheat flour was quite large.

The values obtained by the two different dissolution procedures applied to wheat flour and orchard leaves were in good agreement within one standard deviation, although there was 50% difference in results for the wheat flour (SRM 1567a, Ref. [5]) The relatively large difference between the values obtained by the alkaline and acidic dissolution for the wheat flour may have been due to iodine content being close to the detection limit. The non-destructive analysis was performed for the apple leaves and the results were in good agreement with the RNAA.

Schnetger and Muramatsu determined the iodine concentration in the orchard leaves by ICP-MS and reported 0.17 and 0.26 µg g<sup>-1</sup> (duplicate analysis) [4]. The result obtained by RNAA by Takagi et al. was 0.192±0.010 µg I g<sup>-1</sup> [8]. Shinonaga et al. reported 0.009 µg g<sup>-1</sup> of iodine in the JF-1 sample analysed by RNAA applying an alkaline conversion for sample dissolution [10]. A high precision (RSD 3.4%) was obtained for JF-1 in the present study.

#### 3.5. Concentration of iodine in Austrian cereal grains and precision of analysis

Some of the analytical results for cereal grains are shown in Table I. The detailed data are being published elsewhere [11]. The concentrations of iodine in cereal grains ranged from 0.002 to 0.03 µg g<sup>-1</sup> with an arithmetic mean of 0.0061 µg g<sup>-1</sup>.

TABLE I. ANALYTICAL RESULTS OF IODINE DETERMINATION

Sample	Type	Dissolution <sup>a</sup>	N <sup>b</sup>	Mean (μg g <sup>-1</sup> )	SD <sup>c</sup> (μg g <sup>-1</sup> )
SRM 1567a	Wheat flour	Al <sup>d</sup>	10	0.0017	0.0006
		Ac <sup>e</sup>	3	0.0009	0.0002
		Total <sup>f</sup>	12	0.0015	0.0006
		Ref. <sup>g</sup> [5]		<0.0009> <sup>h</sup>	
SRM 1571	Orchard leaves	Al	2	0.17/0.23	
		Ac	7	0.18	0.02
		Total	9	0.18	0.03
		Ref. [6]		<0.17>	
SRM 1515	Apple leaves	Ac	9	0.32	0.03
		Nd <sup>i</sup>	4	0.29	0.02
		Total	13	0.30	0.03
		Ref. [7]		<0.30>	
JF-1	Volcanic rock <sup>j</sup>	Ac	3	0.0118	0.0004
		Ref. [10]		<0.009>	<0.004>
Austrian cereal grain (Part of results obtained)					
WW– 1	Winter wheat	Al	3	0.0049	0.0002
		Ac	5	0.0059	0.0006
		Total	8	0.0055	0.0007
2	Winter wheat	Ac	3	0.0035	0.0001
3		Al	3	0.0092	0.0011
WR– 1		Ac/Al	2	0.004/0.005	0.0005
2	Winter rye	Al	3	0.0040	0.0004
3		Al	3	0.0022	0.0006
SW– 1	Spring wheat	Al	3	0.0052	0.0006
2		Al	3	0.0048	0.0007
3		Al	3	0.0078	0.0012

<sup>a</sup>Type of dissolution. <sup>b</sup>Number of analysis. <sup>c</sup>Standard deviation. <sup>d</sup>Alkaline dissolution. <sup>e</sup>Acidic dissolution (HNO<sub>3</sub>:HCl:HClO<sub>4</sub>=1:3:3). <sup>f</sup>All data. <sup>g</sup>Reference source. <sup>h</sup>< > Non-certified value. <sup>i</sup>Non-destructive analysis. <sup>j</sup>Mixture of orthoclases and albite.

No characteristic ranges of iodine contents were found for winter wheat, spring wheat, or rye. The concentration of iodine in most of the samples was in the range of several  $\text{ng g}^{-1}$ , and several tens of  $\text{ng g}^{-1}$  of iodine were found only in three samples. The distribution of RSD for iodine concentration below  $10 \text{ ng g}^{-1}$  was 21, 24, 21, 10, and 24% for all data for the ranges of 1 to 10, 11 to 20, 21 to 30, 31 to 40, and > 40% of RSD, respectively.

Johansen and Steinnes [12], and Takagi et al. [8] have obtained the analytical results of 0.0027 (RSD 12%, n=7) and 0.0037 (RSD 16%, n=5) for iodine in wheat flour.

#### 4. CONCLUSION

Concentrations of iodine in cereal grains cultivated in Austria were estimated for the first time in this study. The analytical methods were confirmed by three different procedures (alkaline and acidic dissolution, and non-destructive analysis) using standard reference materials by means of neutron

activation analysis. A rapid and simple acidic-dissolution procedure was demonstrated in this study. The concentration of iodine in the wheat grain ranged from 0.002 to 0.03  $\mu\text{g g}^{-1}$  and the arithmetic mean was 0.0061  $\mu\text{g g}^{-1}$ . Most of cereal grains analysed were at levels of several  $\text{ng g}^{-1}$  of iodine. The comparison among the results obtained by the three analytical procedures, and the agreements of their analytical results, confirm the determined results at low level of iodine in cereal grain, few of which have been reported before. Furthermore the data obtained in this study could be helpful for further study of the ecology of iodine in the environment [13].

## REFERENCES

- [1] WHITEHEAD, D.C., The distribution and transformations of iodine in the environment, *Environ. International* **10** (1984) 321–339.
- [2] MURAMATSU, Y., OHMOMO, Y., Iodine-129 and iodine-127 in environmental samples collected from Tokaimura/Ibaraki, Japan. *Total Environ.* **48** (1986) 33–43.
- [3] HEUMANN, K.G., WEISS, H., Massenspektrometrische Jod-sprenbestimmung in geochemischen Standard-Referenzmaterialien und in Meteoriten, *Fresenius Z. Anal. Chem.* **323** (1986) 852–858.
- [4] SCHNETGER, B., MURAMATSU, Y., Determination of halogens, with special reference to iodine, in geological and biological samples using pyrohydrolysis for preparation and inductively coupled plasma mass spectrometry and ion chromatography for measurement, *Analyst* **121** (1996) 1627–1631.
- [5] ANONYMOUS, Certificate of Analysis, Standard Reference Material 1567a, Wheat Flour, National Institute of Standards and Technology, Gaithersburg, MD (1988).
- [6] ANONYMOUS, Certificate of Analysis, Standard Reference Material 1571, Orchard Leaves, National Institute of Standards and Technology, Gaithersburg, MD (1988).
- [7] ANONYMOUS, Certificate of analysis, standard reference material 1515a, Apple Leaves, National Institute of Standards and Technology, Gaithersburg, MD (1993).
- [8] TAKAGI, H., et al., A simple and rapid method for the determination of iodine in rice samples by radiochemical neutron activation analysis, *Bunsekikagaku* **32** (1983) 512–516. (in Japanese, abstract in English).
- [9] LARSEN, E., LUDWIGSEN, M.B., Determination of iodine in food-related certified reference materials using wet ashing and detection by inductively coupled plasma mass spectrometry, *J. Anal. Atom. Spectrom.* **12** (1997) 435–439.
- [10] SHINONAGA, T., et al., Cl, Br and I in igneous standard rocks, *Chemical Geol.* **115** (1994) 213–225.
- [11] SHINONAGA, T., et al., Determination of iodine in cereal grain and standard reference materials by neutron activation analysis, *Int. J. Environ. Anal. Chem.* (in press).
- [12] JOHANSEN, O., STEINES, E., Determination of iodine in plant material by a neutron-activation method, *Analyst* **101** (1976) 455–457.
- [13] SHINONAGA, T., et al., Transfer of iodine from soil to cereal grains in agricultural areas of Austria, *Sci. Total Environ.* (in press).

## MEASURING ISOTOPIC SIGNATURES IN WATER-SOLUBLE ORGANIC CARBON

R. HOOD, L. MAYR  
Soil Science Unit,  
IAEA Laboratories,  
Seibersdorf, Austria

K. McTIERNAN  
Institute of Grassland and Environmental Research,  
North Wyke Research Station,  
Okehampton, Devon, United Kingdom

### Abstract

Water-soluble organic carbon (WSOC) is reported to be the immediate organic substrate for soil microorganisms. Therefore, we studied WSOC replenishment using the difference in  $^{13}/^{12}\text{C}$  stable isotope ratios of maize (a C4 plant) from that of native C3 vegetation, to determine whether rhizodeposition is a significant source of WSOC. In a second experiment,  $^{13}\text{C}$  values of WSOC were used to study the long term effects of nitrogen and water management on C mineralization. The  $^{13}/^{12}\text{C}$  ratios of WSOC were measured by linking a total organic carbon analyser (TOCA) to a mass spectrometer. In this way, interference from inorganic carbon was removed using the phosphoric acid purge cycle of the TOCA. Carbon dioxide from the samples was channelled from the outflow of the TOCA and cryogenically trapped using liquid  $\text{N}_2$ , prior to introduction into a dual-inlet mass spectrometer. The results from the first experiment suggested that rhizodeposition is not a significant source of WSOC replenishment. Results from the second experiment suggested that long term management affects the  $^{13}/^{12}\text{C}$  isotopic ratios of WSOC.

### 1. INTRODUCTION

Understanding the carbon cycle of the soil is key to managing its organic matter [1]. Many models have been proposed to describe the breakdown of soil organic matter (SOM), but they require an understanding of the turnover and source of definable and measurable carbon pools [2]. Soil organic matter is made up of various definable carbon pools: humus, microbial biomass, water-soluble organic carbon (WSOC), etc., which are present in varying quantities and decompose at different rates. Humus is the largest and most stable carbon pool [3], the soil microbial biomass is the dynamic mediator of many of the biological processes in the soil and is both the source and sink of plant nutrients, and WSOC is thought to be the carbon energy source for the microbial biomass [4].

The composition of organic matter in a particular soil profile reflects biological production, biological and chemical decomposition, chemical and physical adsorption, and transport processes, all of which are sensitive to management. Because water-soluble organic carbon (WSOC) is reported to be the immediate organic substrate for soil microorganisms, we set out to study WSOC replenishment, the mechanisms of which include:

- desorption from soil colloids and soil surfaces,
- exudation from plant roots (rhizodeposition),
- dissolution from litter, SOM, biomass waste products, and dead biomass, and
- hydrolysis of insoluble organic polymers.

Using  $^{13}\text{C}$  stable isotope techniques, we hoped it would be possible to ascertain the dominant process in WSOC replenishment. The aim of our first experiment was to determine whether rhizodeposition is a significant source of WSOC, using the differences in  $^{13}/^{12}\text{C}$  stable isotope ratios of maize, a C4 species with a  $\delta^{13}\text{C}$  of  $-11.75\text{‰}$  compared to SOM with a  $\delta^{13}\text{C}$  of  $-25.28\text{‰}$ , derived from native C3 vegetation with a  $\delta^{13}\text{C}$  in the range of  $-34$  to  $-27\text{‰}$ .

TABLE I. TREATMENTS IN EXPERIMENT 2

Notation	Treatment
ZNU	No fertilizer or slurry N for 30 years, un-drained
Zero nitrogen, undrained	
MND	200 kg N ha <sup>-1</sup> NH <sub>4</sub> NO <sub>3</sub> for over 15 years, drained
Mineral nitrogen, drained	
ZND	No fertilizer or slurry N for 30 years, drained
Zero nitrogen, drained	
HNU	200 kg N ha <sup>-1</sup> NH <sub>4</sub> NO <sub>3</sub> for over 15 years; slurry between 1992 and 1996, undrained
High nitrogen, undrained	

In a second experiment, <sup>13</sup>C values of WSOC were used to study the long term effect of nitrogen and water management on carbon mineralization.

### 3. MATERIALS AND METHODS

Experiment 1 was conducted at the IAEA laboratories, Seibersdorf, Austria. The soil is a calcareous clay loam, Typic Eurocrepts (FAO), pH 8.2, organic matter 40 g kg<sup>-1</sup> soil [5]. Immediately after the harvest of maize (*Zea mays*), 12 cm long × 8 cm diameter soil cores were taken from single-season maize plots [previously sown to ryegrass (*Lolium perenne*)], which had received 200 kg N ha<sup>-1</sup> in the form of soybean residues, urea, or sewage sludge. Zero-N plots were also sampled in addition to bare soil outside the maize-growing area [inter-plot (mid) and 1.5 m from the maize plot (out)].

Experiment 2 was sited on the grassland steer-grazed 1-ha lysimeter plots of the Rowden Moor drainage experiment [6]. The soil is clayey and non-calcareous (Dystic Gleysol, FAO) [7,8]. Four 20-cm soil cores were taken from each of the treatment plots described in Table I, and were cut into 5-cm segments. Sub-samples were taken, dried at 70°C, ground and analysed, using the IRMS Optima Micromass system (Micromass UK, Wythenshaw) linked to a Carlo Erba Strumentazione nitrogen-carbon analyser 1500 combustion unit (Milan, Italy).

Water-soluble organic carbon was extracted with deionized distilled water from freshly sieved soil (5:1 ratio), by rotary shaking at 250 rpm for 1 h. Samples were centrifuged at 10,000 rpm for 20 min and the supernatants were passed through a 0.45-μm filter [9].

Measurement of <sup>13</sup>C in the soluble carbon was made by connecting the total organic carbon analyser (TOC-Teckmar-Dohrmann) to the Optima mass spectrometer. The principle of the TOCA, in soluble organic carbon analysis mode, is that the sample is purged with phosphoric acid to eliminate all inorganic carbon as CO<sub>2</sub>, which is vented to waste; a sub-sample is taken and a peroxide-UV oxidation breaks down all the organic carbon to CO<sub>2</sub>, measured using infra-red gas analysis. By connecting the detector outflow pipe to a cryogenic trap, it was possible to cryofocus the carbon dioxide and determine the <sup>13</sup>C/<sup>12</sup>C ratios of the soluble organic carbon in dual inlet mode, using a reference gas. IAEA standards were used to calibrate the gas.

## 4. RESULTS

### 4.1. Experiment 1

Maize cover had no significant ( $P < 0.05$ ) effect on the isotopic signature of the WSOC or the concentration of WSOC (Figs. 1 and 2). The  $\delta^{13}\text{C}$  of the zero-N plot reflected the  $\delta^{13}\text{C}$  of the soil organic matter. The organic amendments did not significantly alter the <sup>13</sup>C/<sup>12</sup>C ratios of the WSOC pool. However, it appeared that the organic amendments may influence the <sup>13</sup>C/<sup>12</sup>C ratio of the WSOC pool, as the <sup>13</sup>C/<sup>12</sup>C of the WSOC tended towards the <sup>13</sup>C/<sup>12</sup>C value of the organic amendment and



there was a higher concentration of WSOC in the treatment with soybean residues added. There was a highly significant linear relationship ( $P < 0.001$ ,  $r^2 = 0.67$ ) between concentration of WSOC and the  $\delta^{13}\text{C}$  of the WSOC (data not shown).

## 4.2. Experiment 2

There were consistent differences in  $\delta^{13}\text{C}$  of the WSOC between treatments (Fig. 3). The  $\delta^{13}\text{C}$  of the WSOC generally decreased significantly with decreasing depth in all treatments. Only in the MND treatment was the  $\delta^{13}\text{C}$  of the WSOC in the 0–5-cm layer significantly more negative than those of similar depth in other treatments. Also, interestingly, in the ZNU treatment there was a more defined drop in the  $\delta^{13}\text{C}$  of the WSOC between the surface layer and the deeper layers. The concentration of WSOC also generally decreased with depth and the MND treatment had the lowest WSOC concentration at the surface layer (Fig. 4).

There was a highly significant ( $P < 0.001$ ) correlation between the  $\delta^{13}\text{C}$  of the WSOC and the  $\delta^{13}\text{C}$  of the total organic carbon (TOC) of the soil (Fig. 5a) and between  $\delta^{13}\text{C}$  of the WSOC and the concentration of total soil organic carbon (Fig. 5b). As previously observed by Balesdant [10] and Warren and Meridith [11] there was a highly significant correlation between % organic carbon and the  $\delta^{13}\text{C}$  of the organic carbon of the soil ( $r^2 = 0.81$ ) (Fig. 5c).

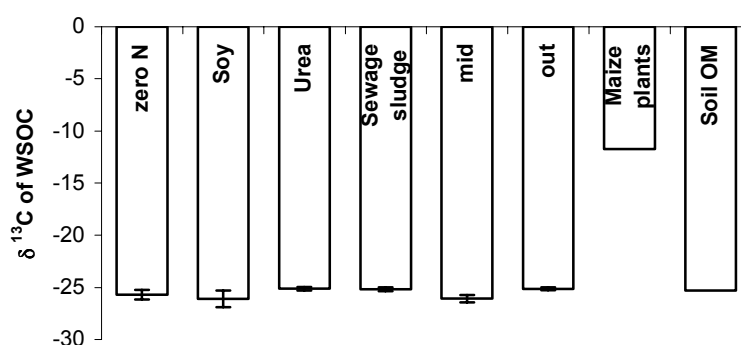


FIG. 1.  $\delta^{13}\text{C}$  of WSOC in Experiment 1.

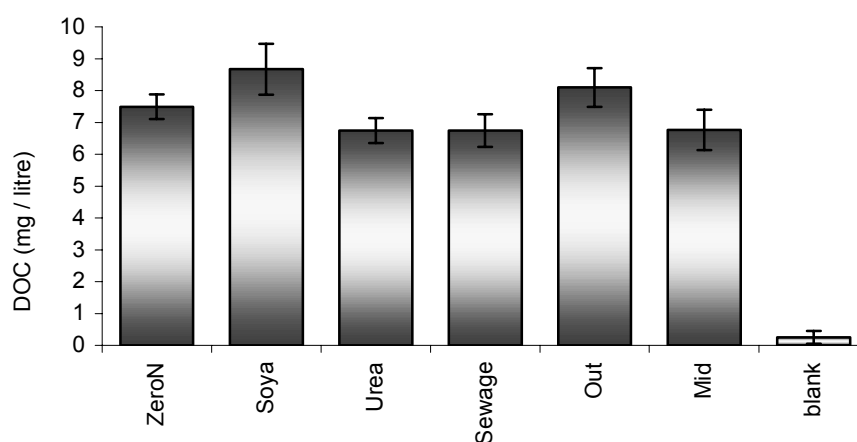


FIG. 2. DOC concentration of WSOC in Experiment 1.

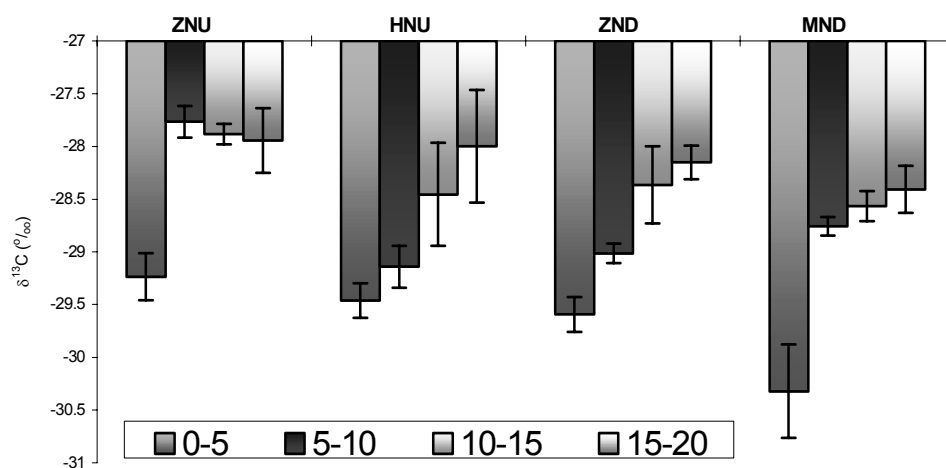


FIG. 3.  $\delta^{13}\text{C}$  of WSOC in Experiment 2.

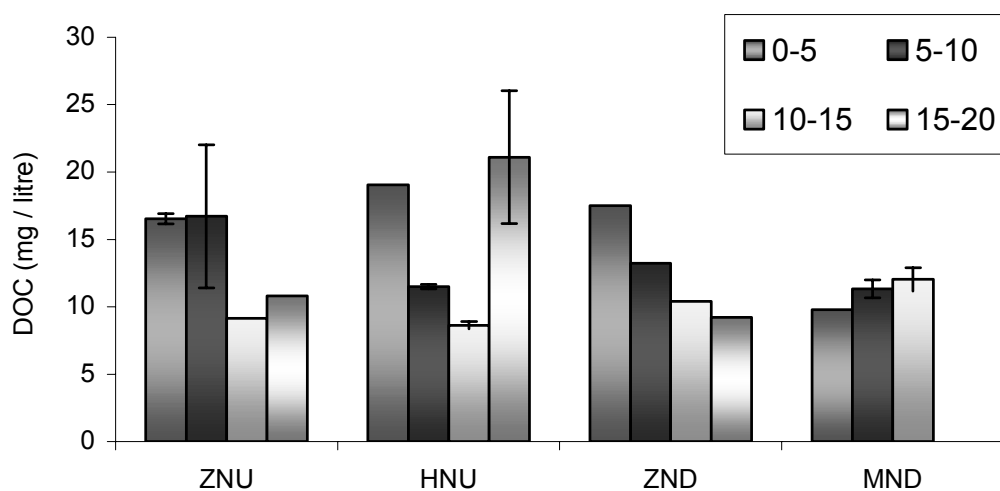


FIG. 4. DOC concentration of WSOC in Experiment 2.

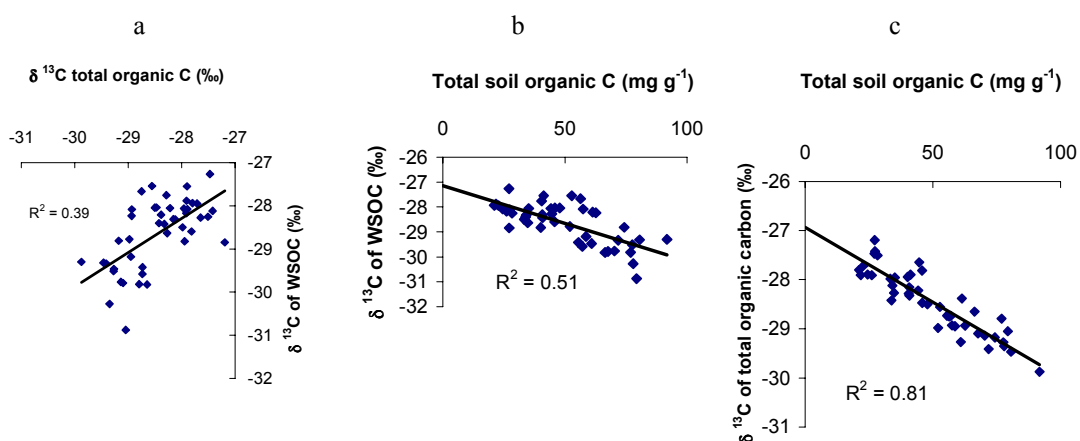


FIG. 5. (a)  $\delta^{13}\text{C}$  of TOC against  $\delta^{13}\text{C}$  of WSOC, (b)  $\delta^{13}\text{C}$  of WSOC against TOC concentration, (c)  $\delta^{13}\text{C}$  of TOC against TOC concentration.

## 5. DISCUSSION

From the data it may be inferred that there was little or no input from the maize crop to WSOC pool at harvest. Data from Gregorich et al. [12] suggest it is theoretically possible to detect the shift in the signal of  $\delta^{13}\text{C}$  in the WSOC pool after 1 year of maize cropping on a site previously cropped to C3 plants. In this experiment, sampling was done at the end of the season and it likely that any potential contribution from the maize crop had been utilized and respired off earlier in the growing season. Indeed, previous studies in grasslands have shown that WSOC is made up of the more recalcitrant forms of carbon, humic and fulvic acids [13,14]. Rhizodeposition from maize is in the form of exudates, secretions, mucilages that are more rapidly utilized by the microbial biomass, as well as sloughed root-cap cells, and decaying root hairs [15]. Therefore, if rhizodeposition contributes significantly to the WSOC pool, one would expect to detect some evidence of it in the  $\delta^{13}\text{C}$  signal. The  $\delta^{13}\text{C}$  of the WSOC reflected the  $\delta^{13}\text{C}$  of the TOC pool, implying that the source of WSOC was the TOC pool; the strong relationship between  $\delta^{13}\text{C}$  signal and C concentration of the soil WSOC pool also suggested this. The concentration of WSOC was slightly higher and the  $\delta^{13}\text{C}$  value slightly more negative in the soybean treatment, suggesting that there may be an influence of residue addition on the WSOC pool.

In Experiment 2, the upper layers of soil had significantly more-negative  $\delta^{13}\text{C}$  signals than did the lower layers, and the  $\delta^{13}\text{C}$  of the upper layer WSOC pool was significantly more negative in the MND treatment, indicating a greater contribution of C from the grass, at 31.73‰. The  $\delta^{13}\text{C}$  of the WSOC reflected the  $\delta^{13}\text{C}$  of the TOC pool, strongly suggesting that the source of WSOC was the TOC pool. Again the  $\delta^{13}\text{C}$  of the WSOC was strongly correlated with the TOC concentration, implying that the source of WSOC was the TOC pool. There was a significant linear increase in ( $r^2 > 0.9$ , data not shown) in the  $\delta^{13}\text{C}$  of the TOC pool with depth in all treatments. This was previously observed by Balesdent et al. [10], which he attributed to three factors:

- discrimination against  $^{13}\text{C}$  in decomposition,
- discrimination against  $^{13}\text{C}$  in mineralization, and
- change in the atmospheric  $\text{CO}_2$  concentration, the mean value having decreased by 1.2‰ over the last 130 years, due to the widespread use of fossil fuels which have a  $\delta^{13}\text{C}$  signal of about – 26‰ [16].

However, the changes in  $\delta^{13}\text{C}$  signals in Experiment 2 fell from 30.5‰ to as low as 27.8‰ in both the TOC and WSOC pools, greater than the 1.2‰ shift caused by burning of fossil fuels. It is likely that the increased  $^{13}\text{C}$  with depth is due to the older, more decomposed nature of the organic matter in the deeper soil layers.

## 6. CONCLUSIONS

Linking the total organic carbon analyser to the mass spectrometer proved a useful way to study the  $\delta^{13}\text{C}$  of WSOC. The use of the differences in  $\delta^{13}\text{C}$  of C3 and C4 plants made it possible to determine the source of WSOC replenishment and to conclude that the most likely source of WSOC was the soil organic matter. However, it was also noted that, as the WSOC is such a dynamic pool, studies should be undertaken that cover the entire growing season to assess the seasonal contribution from rhizodeposition, as these inputs could be ephemeral but significant. In the grassland system, it was also concluded that the most likely source of WSOC was the soil organic matter. The  $\delta^{13}\text{C}$  of the TOC pool probably reflected the age and stage of decomposition of the TOC pool.

## REFERENCES

- [1] BALESDENT, J., Analysis of soil organic matter dynamics using carbon isotopes. Les isotopes du carbone et la dynamique des matières organiques des sols, Cahiers Agricultures 7 (1998) 201–206.

- [2] WHITMORE, A. P., et al., "Simulating trends in soil organic carbon in long term experiments using the Verberne/MOTOR model", *Evaluation and Comparison of Soil Organic Matter Models* (SMITH, P., et al., Eds.), *Geoderma* **81** (1997), 137–151.
- [3] JENKINSON, D.S., The turnover of organic carbon and nitrogen in soil, *Phil. Trans. Royal Soc.* **B329** (1990) 361–368.
- [4] ZAK, D.E., et al., Carbon and nitrogen cycling during old field succession: Constraints on plant and microbial productivity, *Biogeochem.* **10** (1990) 111–129.
- [5] Hood, R.C., et al., A comparison of direct and indirect  $^{15}\text{N}$  isotope techniques for estimating crop N uptake from organic residues, *Plant Soil* **208** (1999) 259–270.
- [6] TYSON, K.C., et al., Comparison of crop yields and soil conditions during 30 years under annual tillage or grazed pasture, *J. Agric. Sci.* **115** (1990) 29–40.
- [7] SCHOLEFIELD, D., et al., Nitrate leaching from grazed grassland lysimeters: effects of fertilizer input, field drainage, age of sward and patterns of weather, *J. Soil Sci.* **44** (1993) 601–613.
- [8] ARMSTRONG, A.C., GARWOOD, E.A., Hydrological consequences of artificial drainage of grassland, *Hydrological Processes* **5** (1991) 157–174.
- [9] MCGILL, W.B., et al., Dynamics of soil microbial biomass and water-soluble organic C in Breton L after 50 years of cropping to two rotations, *Can. J. Soil Sci.* **66** (1986) 1–19.
- [10] BALESSENT, J., et al., Natural  $^{13}\text{C}$  abundance, a tracer of soil organic matter dynamic studies. *Soil Biol. Biochem.* **19** (1987) 25–30.
- [11] WARREN, G.P., MERIDITH, J.A., "Isotopic composition of organic carbon in some soils of Semiarid Africa", *Carbon and Nutrient Dynamics in Natural and Agricultural Tropical Ecosystems* (BERGSTRÖM L., KIRCHMANN, H., Eds.), CAB International, Oxford (1998) 23–32.
- [12] GREGORICH, E.G., et al., Elucidation of the source and turnover of water soluble and microbial biomass carbon in agricultural soils, *Soil Biol. Bioch.* **32** (2000) 581–587.
- [13] McTIERNAN, K.B., et al., Short term changes in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures of water discharged from grazed grasslands, *Rapid Comm. Mass Spectrom.* **13** (1999) 1803–1807.
- [14] BOL, R., et al., Tracing dung-derived carbon in temperate grassland using  $^{13}\text{C}$  natural abundance measurements, *Soil Biol. Biochem.* **32** (2000) 1337–1343.
- [15] SWINNEN, J., et al., Carbon fluxes in the rhizosphere of winter wheat and spring barley with conventional vs integrated farming, *Soil Biol. Biochem.* **27** (1995) 811–820.
- [16] FRIEDLI, H., et al., Ice core record of the  $^{13}\text{C}/^{12}\text{C}$  ratio of atmospheric  $\text{CO}_2$  in the past two centuries, *Nature* **324** (1996) 237–238.



## **POSTER SESSION**



# USE OF NUCLEAR TECHNIQUES TO EVALUATE MANAGEMENT PRACTICES FOR COMMON BEAN: A NEW P FERTILIZER BASED ON PARTIALLY SOLUBILIZED ROCK PHOSPHATE

A. GARCÍA, G. HERNÁNDEZ, A. NUVIOLA  
Soil Institute, Havana, Cuba

D. MONTANGE  
Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD),  
Montpellier, France

J.J. DREVON  
Institut National de Recherche Agronomique (INRA),  
Montpellier, France

Experiments were carried out in the laboratory, glasshouse, and field, in a Rhodic ferralsols soil. In all experiments the treatments were: a Cuban rock phosphate (RP), Cuban RP partially solubilized (50%) with  $H_2SO_4$  (PSRP), triple superphosphate (TSP), and a control. The isotopic exchangeable kinetic (IEK) method showed that all of the treatments increased the concentration of P in the soil solution ( $C_p$ ) (determined by the green malachite method), the isotopically exchangeable P ( $E_1$ ), and the values of the kinetic indexes according to the degree of solubilization of each P source (Table I).

The pot experiment, carried out using the isotope-dilution (ID) method, showed that TSP and PSRP were more effective than RP in terms of dry matter, P yields, and fertilizer-use efficiency (FUE) of common bean. The P fractions derived from fertilizers were calculated from the IEK experiment using Fardeau's formulas:  $\%PdfF = 100 [E_{1F} - E_{1C}]/E_{1F}$  and  $\%PdfF = 100 [C_{p1F} - C_{p1C}]/C_{p1F}$  [1] and were related by linear regression with those obtained from the ID experiment (Table II). The FUE values were calculated from the regression equations and the values of P yields obtained from the ID experiment (Table III).

The FUE values, calculated by the ID method and from Fardeau's indexes, were closely related by linear regression, where the independent terms were near zero and the regression coefficients were near 1, indicating the possibility of estimating the P-FUE from the IEK method or simply by the accurate determination of P in the soil solution.

TABLE I. RESULTS OF THE IEK EXPERIMENT

Treatment	$r_1/R$	$C_p$ ( $\mu g P mL^{-1}$ )	$E_1$ ( $\mu g P g^{-1}$ )	n	Km ( $min^{-1}$ )	Tm (min)	Fm [ $g P (\mu g min)^{-1}$ ]
Control	0.044	0.020	4.6	0.394	1092.7	$9.2 \cdot 10^{-4}$	218
RP	0.049	0.025	5.1	0.351	1892.1	$5.3 \cdot 10^{-4}$	473
PSRP	0.059	0.036	6.1	0.391	544.2	$1.8 \cdot 10^{-3}$	196
TSP	0.060	0.042	7.0	0.349	1906.2	$9.0 \cdot 10^{-4}$	465

TABLE II. RESULTS OF THE REGRESSION ANALYSIS

Cuts	Equation	$R^2$
First	$\%PdfF_{ID} = -14.7 + 3.48\%PdfF_{IEK E1}$	0.92
	$\%PdfF_{ID} = -37.5 + 2.63\%PdfF_{IEK Cp}$	0.98
Second	$\%PdfF_{ID} = -2.6 + 2.83\%PdfF_{IEK E1}$	0.94
	$\%PdfF_{ID} = -20.6 + 2.12\%PdfF_{IEK Cp}$	0.99



TABLE III. FERTILIZER USE EFFICIENCY (FUE, %) CALCULATED FROM THE VALUES OF %PDFF OBTAINED BY THREE DIFFERENT METHODS

Treat.	First cut			Second cut			Total		
	ID	IEK E <sub>1</sub>	IEK C <sub>p</sub>	ID	IEK E <sub>1</sub>	IEK C <sub>p</sub>	ID	IEK E <sub>1</sub>	IEK C <sub>p</sub>
RP	0.2	0.3	0.2	0.7	0.8	0.7	0.9	1.1	0.9
PSRP	3.3	2.7	3.0	4.8	4.2	4.6	8.1	6.9	7.6
TSP	5.0	5.2	5.2	7.4	7.9	7.6	12.4	13.1	12.8

The use of all of the P fertilizers increased with time, and RP reached 1% due to the soil pH value (neutral).

In the field experiment genotype, cultivar BAT 477 of common bean and rhizobial strain CIAT 899 were used. The experimental results (means for 2 yrs) and the economic evaluation were in agreement with those from the IEK and ID experiments, where TSP > PSRP > RP > control (Fig. 1).

A second field experiment was carried out in order to validate the new phosphate fertilizer based on partially solubilized RP, with two common-bean genotypes (BAT 477 and DOR 364) and two rhizobial strains (CIAT 899 and 6bIII). The P source in all of the treatments was PSRP so the treatment (PSRP and AI treatments, respectively) common to both field experiments allowed direct comparisons between them. Experimental results (means for 2 years) and economic evaluations are shown in Fig. 2.

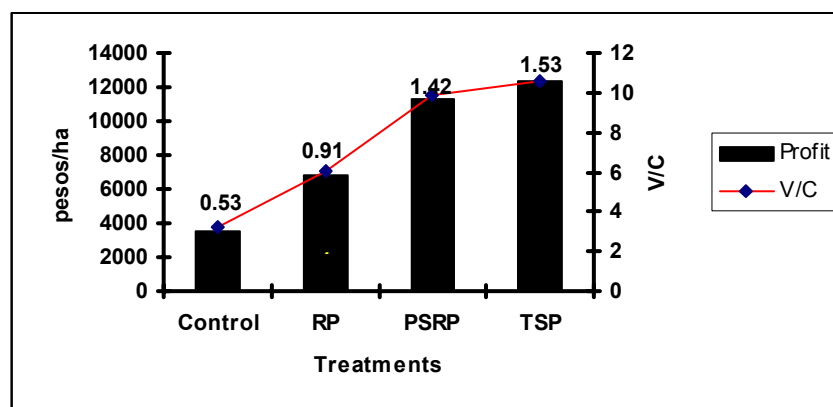


FIG. 1. Grain yields, economic profit, and value/cost ratio from the P-fertilizer experiment (grain yield values are on the bars).

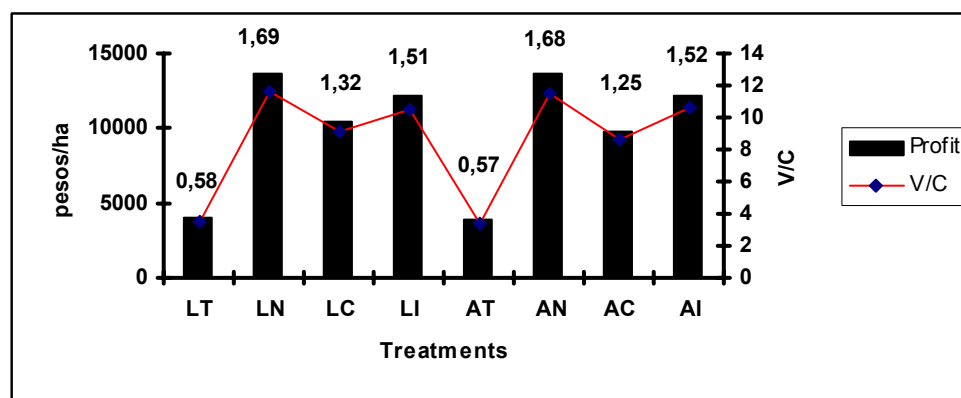


FIG. 2. Grain yields, economic profit, and value/cost ratio validation experiment for a new P fertilizer based on partially solubilized RP (grain yields values are on the bars) (L: cv. DOR 364; A: cv. BAT 477; T: Control; N: N fertilizer; C: strain 6bIII; I: strain CIAT 899.)

In terms of economic benefit, the new P fertilizer (PSRP) was less expensive than imported TSP, as it is produced with local RP and its use can produce financial returns between 9,842 and 13,689 pesos/ha, depending on the common-bean genotype used.

### **ACKNOWLEDGEMENTS**

This work was partially supported by FAO/IAEA Division of Nuclear Techniques in Food and Agriculture (TC Project CUB/5/015). The authors thank Mr. F. Zapata and members of the IAEA Soil Science Unit, Seisberdorf, Austria, for technical assistance, also Mr. Binh Truong and Ms. Claire Chevassus of CIRAD-CA, Montpellier, France.

### **REFERENCE**

- [1] INTERNATIONAL ATOMIC ENERGY AGENCY, Final Report of the FAO/IAEA Co-operative Research Project on the Use of Nuclear and Related Techniques for Evaluating the Agronomic Effectiveness of Phosphate Fertilizers, in Particular Rock Phosphate, Joint FAO/IAEA Div. of Nuclear Techniques in Food and Agriculture, Soil and Water management & Crop Nutrition Section, Vienna (1999).

## EVALUATION OF THE EFFECTS OF IRRADIATED SEWAGE SLUDGE ON PLANT GROWTH

S. LOPEZ, J.P. BONETTO, N. BARBARO

Comisión Nacional de Energía Atómica (CNEA),  
Buenos Aires, Argentina

Experiments were carried out to estimate and compare nutrient uptake from irradiated sewage sludge and from two chemical fertilizers (triple superphosphate and ammonium sulphate). The sewage sludge was obtained from a treatment plant at Tucumán, Argentina, and was treated by  $\gamma$ -irradiation (dose: 3 kGy). It was added to three soils from Tucumán, with pH values between 6.4 and 6.6. Soils I and III had similar organic matter content (2.2 and 2.6%, respectively) and extractable P Bray (3.44 and 3.40  $\mu\text{g mL}^{-1}$ , respectively). Organic matter in Soil II was 3.8% and extractable P was 12.1  $\mu\text{g mL}^{-1}$ . The sewage sludge had a pH of 6.6, organic matter content of 22%, total N of 1.0%, total P of 0.7%, and a C:N ratio of 13.

Annual ryegrass (*Lolium multiflorum*) was grown under greenhouse conditions, in pots containing 1 kg of soil, with three levels of sewage sludge (Table I). Treatments with unlabelled superphosphate (TSP) and ammonium sulphate (AS) were included to assess the effectiveness of sewage sludge as a fertilizer (Table I).

Three harvests were made, at 40, 81, and 125 days from seeding. The availability of P and N from the sewage sludge was studied by isotope-dilution, using  $^{32}\text{P}$ -superphosphate and  $^{15}\text{N}$ -ammonium sulphate. Solid labelled fertilizers were added to the pots with and without sewage sludge. The amounts of N and P uptake was calculated from the comparison of  $^{15}\text{N}$  abundance or  $^{32}\text{P}$  specific activity in plants grown in controls and sewage sludge (SS) treatments. All treatments were replicated three times.

Accumulated dry weight increased with the addition of sewage sludge (Fig. 1). The medium rate of sewage sludge produced a higher increase than the others. However, when all soils were considered, the only significant effect of fertilizer was with ammonium sulphate (AS) (Table II).

TABLE I. AMOUNTS OF P AND N APPLIED IN SEWAGE SLUDGE (SS), TRIPLE SUPERPHOSPHATE (TSP), AND AMMONIUM SUPHATE (AS)

Rate	Treatment					
	Control	SS1	SS2	SS3	TSP	AS
Pot (mg P)	0	11.5	23.1	34.6	23.1	
Pot (mg N)	0	15.4	30.8	46.1		30.8
Field (kg/ha)	0	3,000	6,000	9,000	225	283
Field (kg P or N/ha)					45	60

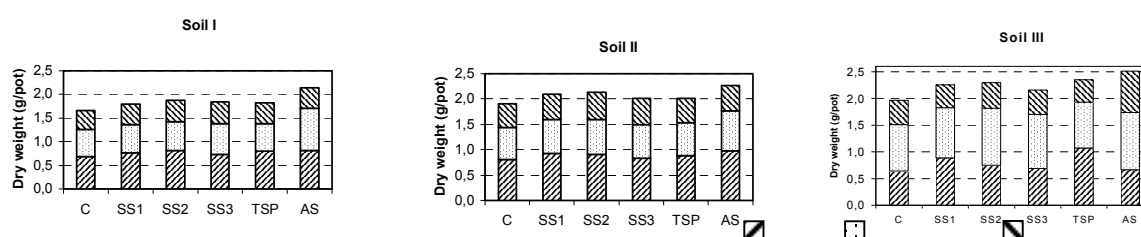


FIG. 1. Dry weight accumulation of ryegrass in three soils, = 40, = 81, and = 125 days.

**TABLE II. YIELD AVERAGES (g dry matter pot<sup>-1</sup>) IN SOILS AND TREATMENTS**

Soil			Treatment					
I	II	III	Control	SS1	SS2	SS3	TSP	AS
1.79a <sup>*</sup>	2.02b	2.21c	1.84a	2.05b	2.10b	2.01b	2.06b	2.30c

<sup>\*</sup>Means followed by different letters are significantly different ( $P > 0.05$ ) [1].

Treatments that received the same amount of P as sewage sludge or triple superphosphate (SS2 and TSP) showed similar yields. Amounts of N and P taken up were higher when ammonium sulphate was applied.

In all soils, P-concentration and P-yield values in the sewage sludge treatments were higher than in the control without fertilizer. The percentages of P derived from sewage sludge treatment SS2 (Pdfss) were: 16, 17, and 25% in soils I, II, and III, respectively. In soil I the addition of sewage sludge produced consistent increases in N and P plant content during the first and second growing periods.

The uptake of N in soils II and III could not be explained so easily. In soil II there was no significant difference in total N content between treatments except with ammonium sulphate. In soil III, there was a different pattern of N uptake. The plants that were harvested first showed N contents inversely related to the rate of sewage sludge applied. During the second period, the N content in plants increased as the amount of sewage sludge increased, but it was less than the N content in control plants. This behaviour was related to the changes in availability of soil N due to immobilization and mineralization affected by the addition of the sewage sludge. Nitrogen from sewage sludge was scarcely available to plants and the uptake of soil N was reduced in some cases. As a consequence, the application of isotopic methodologies to estimate the recovery of N showed some difficulties. In soil I the percentage of N derived from sewage sludge (Ndfss) applied in treatment SS2 was 5%. The Ndfss values in the other two soils were also low, as was expected because of low uptake of total N. But, it was not possible to calculate this value for all of the replications of each treatment. Problems related to the use of <sup>15</sup>N techniques when an inorganic N source and organic residues are simultaneously added to soils have been reported by Hood et al. [2].

We conclude that the medium rate of sewage sludge (6 t ha<sup>-1</sup>) was as effective as TSP in promoting plant growth. Greater availability of both N and P from sewage sludge may be achieved with longer growing periods, especially in relation to N uptake. New approaches for evaluating organic residues are needed.

We are now studying the accumulation of heavy metals and micronutrients in these soils to complete the evaluation of the effects of sewage sludge addition.

## ACKNOWLEDGEMENTS

This research was partly funded by FAO/IAEA, Project ARG8/012. We thank J. Graiño for irradiating the sewage sludge. Also, we thank F. Zapata and S. Urquiaga for their help and comments relating to the design of the experiments.

## REFERENCES

- [1] SAS INSTITUTE INC., SAS User's Guide, Version 6.08, SAS Inst., Cary, NC (1989).
- [2] HOOD, R.C., et al., A comparison of direct and indirect <sup>15</sup>N isotope techniques for estimating crop N uptake from organic residues, *Plant Soil* **208** (199) 259–270.

## ADDITIONAL ADVANTAGES OF IRRADIATION OF SEWAGE SLUDGE FOR AGRICULTURE USE

C. MAGNAVACCA, J.G. GRAIÑO  
Comisión Nacional de Energía Atómica (CNEA),  
Ezeiza, Argentina

The agricultural use of sewage sludge produced in wastewater-treatment plants, to increase soil fertility and crop yields is widely known, but is limited by the presence of pathogenic microorganisms, heavy metals, and toxic chemicals. The irradiation of sludges to eliminate pathogens has been successfully developed. Nevertheless, commercial-scale projects are still not promoted for economic reasons and due to public concerns.

In this paper we report additional advantages of gamma irradiation over other methods of disinfection, i.e. composting, and biological treatments in general:

- decomposition of pesticides in the sludge,
- release of mineral N, thus increasing N availability for plants,
- killing of weed seeds,
- viscosity decrease that helps movement through pipes for management and application.

Anaerobically digested sewage sludges produced at the Wastewater Treatment Plant of Tucuman City were irradiated with  $^{60}\text{Co}$  gamma sources at the SemiIndustrial Irradiation Plant in the Ezeiza Atomic Centre. The absorbed irradiation doses were within the range of 3.5 kGy for liquid sludges and 6.5 kGy for dried sludges, as recommended for disinfection.

Much published work, chiefly with electron beam accelerators, has describe the decomposing effects of ionizing radiation on hazardous organic pollutants in surface or drinking water [1]. There have been a few reports on the decomposition of sludge-borne chemicals by gamma radiation. Organic toxicants that have been detected in Tucuman's municipal sludge include chlorpyrifos, heptachlor, lindane, and phenoxyacids; all were decreased by gamma radiation. A gas chromatograph-mass spectrometer was used to examine sludge and artificially polluted water extracts. Although the effectiveness of radiation was lower in sludge than in aqueous solution, the irradiation treatment appeared to accelerate decomposition of the toxic substance.

Ammonium-N and nitrate-N contents of irradiated and non-irradiated sludges were analyzed by Bremner's methods. The ammonium released by irradiation (Fig. 1) was 2.6% of total N, both in liquid and in dried sludge samples. The  $^{15}\text{N}$ -dilution technique in a greenhouse experiment with ryegrass was used to compare N uptake from irradiated sludge with respect to non-irradiated sludge. Nitrogen uptake and biomass yield were higher with irradiated sludge, in agreement with other work in N-responsive conditions with crops not inhibited by ammonium [2].

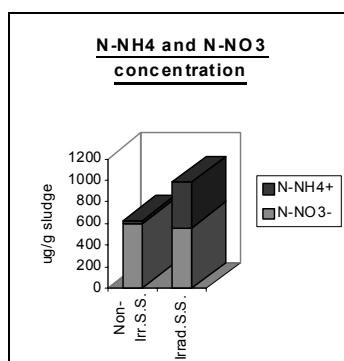


FIG. 1. The effect of irradiation on nitrate and ammonium content of sewage sludge.

TABLE I. EFFECTS OF TREATMENTS ON N-UPTAKE CHARACTERISTICS

Treatment	%Ndff <sup>a</sup>	%N	N yield (mg/pot)	% fertilizer utilized
Control	22	4.83±0.09	43.3	
Urea (100 mg/kg)	10	4.84±0.07	45.6	5.7
S.S. (100 mg/kg)	19	4.81±0.07	34.1	1.3
Irr. S.S. (100 mg/kg)	18	4.99±0.09	47.4	2.1

<sup>a</sup>Nitrogen derived from fertilizer.

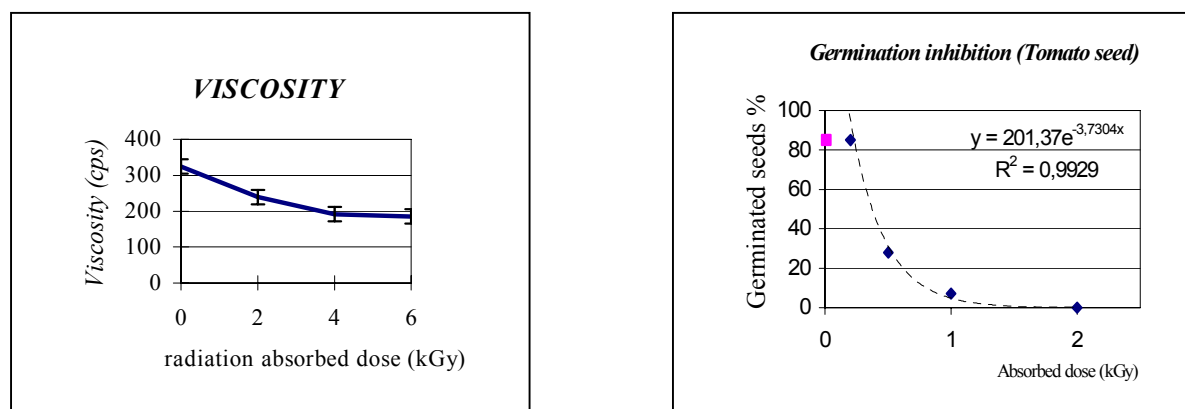


FIG. 2. Effects of absorbed radiation on viscosity of sewage sludge (left) and on germination of seeds of tomato.

Viscosity was measured with a Brookfield viscometer in non-irradiated and irradiated samples with increasing absorbed doses. It was verified that irradiation causes a viscosity decrease (30% lower) with the dose applied for disinfection (Fig. 2). The settling velocity as well as the specific filtration resistance were concomitantly diminished [3]. These observations imply a positive change helping to avoid blockage of pipeworks

Four species of seeds most commonly found in the sludges (tomato, melon, calabash, squash) were irradiated with increasing doses, and percent germination tested. The inactivating effect of irradiation was verified with lower absorbed radiation doses than those applied for disinfection (Fig. 2). The effect fits an exponential function of percentage of germinated seeds vs. radiation dose, varying with the characteristic species radioresistance [4].

### ACKNOWLEDGEMENTS

Part of this work was performed within the framework of an FAO/IAEA Coordinated Research Project and an IAEA Technical Cooperation Project.

### REFERENCES

- [1] GETOFF, N., Decomposition of biologically resistant pollutants in water by irradiation, *Radiat. Phys. Chem.* **35** (1990) 432–439(1990).
- [2] WEN, G., et al., Evaluation of nitrogen availability in irradiated sewage sludge, sludge compost and manure compost, *J. Environ. Qual.* **24** (1995) 527–534.
- [3] GRAÍÑO, J.G., MAGNAVACCA, C., “Sewage sludge irradiation project in Argentina”, *Environmental Applications of Ionizing Radiation* (COOPER, W.J. et al., Eds.), John Wiley and Sons, New York (1998).
- [4] KUMAR, N., MALIK, S.S., Effect of gamma-radiation on germination and seedling growth, *International Rice Research Newsletter* **11** (1986) 6.

## GROSS MINERALIZATION FROM PLANT RESIDUES USING CROSS-LABELLING

C.C. VIDELA

Universidad Nacional de Mar del Plata (UNMdP),  
Balcarce, Argentina

R.C. HOOD

IAEA Soil Science Unit,  
Seibersdorf, Austria

Three greenhouse experiments were carried out to measure gross mineralization rate (GMR), inorganic N dynamics, and plant-N uptake from soybean residues and fertilizer N/residue interactions. The soil was a sandy loam from the Krumbach region of Austria (OC: 9.9 g kg<sup>-1</sup>, total-N: 1.11 g kg<sup>-1</sup>, CEC: 7.53 cmol(+) kg<sup>-1</sup>, pH (1:2.5 water) 7.6, Olsen-P: 12.4 µg g<sup>-1</sup>).

**Gross mineralization** was measured using <sup>15</sup>N-dilution and cross-labelling techniques. Soil was mixed with <sup>15</sup>N-labelled or unlabelled soybean residues and packed into PVC columns, which were injected with 20 mg N kg<sup>-1</sup> soil, in the form of either labelled (10 atom % <sup>15</sup>N excess.) or unlabelled NH<sub>4</sub>NO<sub>3</sub>, 11, 15 and 22 days after residue incorporation (Table I). Columns were sampled 2 and 4 days after injection, and analysed for water content, inorganic N, and the <sup>15</sup>N-enrichment of inorganic-N pools.

Extracts were prepared for <sup>15</sup>N analysis using micro-diffusion [1] and measured using a C-N analyser (Carlo Erba Strumentazione, Milan, Italy) linked to an isotope ratio mass-spectrometer (IRMS) Optima Micromass system (Micromass UK, Wythenshaw). The GMRs were calculated using the equations of Barraclough et al. [2] and the proportion of GM derived from residues ( $\alpha$ ) using the equations of Watkins and Barraclough [3].

**Inorganic N dynamics experiment.** One-kg pots of soil were prepared incorporating labelled soybean residues at rates shown for treatments 1 to 4 (Table I). The soils were sampled 7, 14, 21 and 28 days after residue incorporation to determine the inorganic N.

**Plant N uptake experiment.** Two-kg pots of soil were prepared incorporating labelled soybean residues at rates for treatments 1 to 4 and sown with 0.5 g ryegrass (*Lolium perenne* L.) seeds. After 28 days, shoots and roots were harvested, and dry matter, %N, and <sup>15</sup>N-enrichments determined.

**GM experiment.** Treatment 1 showed a linear increase in GMR over time (Table II). Data from the first and second injections showed that the GMRs increased linearly with increasing N residue incorporation rate ( $R^2=0.94$ ), also reflected in the  $\alpha$  values ( $R^2=0.98$ ). In all of the residue treatments GMRs were maximal at the second injection, however  $\alpha$  values were minimal. With the last injection, GM decreased linearly with increasing residue addition ( $R^2=0.93$ ); the 300N treatment showing the lowest GMR. These results suggest preliminary utilization of a labile N pool or soluble C source supplied by the residue, followed by a reduced GMR due to residues. The beauty of the GM and  $\alpha$  measurements is that they provide a temporal study of dynamics of residue breakdown.

In the **inorganic N experiment**, the dominant N pool was nitrate, suggesting a rapidly nitrifying soil. The Ndfres data (not shown) indicated that residue addition initially increased the inorganic-N pool from mineralization of residues. The results showed a rapid mineralization of residues over the first 7 days, again suggesting an easily decomposable labile N pool.

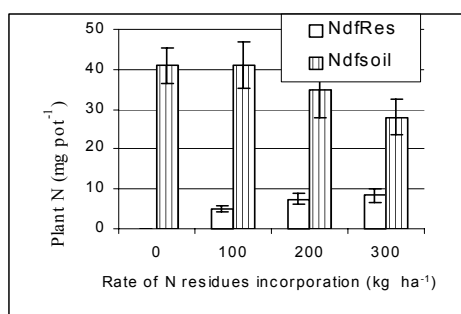
**Plant-N uptake.** from the residue increased with increasing residue addition (Fig. 1). Total plant-N uptake was lowest in the 300N treatment, and Ndfsoil decreased with increasing residue addition (Fig. 1); care must be taken when interpreting such data due to errors associated with pool substitution.

TABLE I. TREATMENTS FOR THE GROSS MINERALIZATION EXPERIMENT

Treatment number and description	Form of label injected
1 Soil only (0N)	$^{15}\text{NH}_4\text{NO}_3$
2 Soil + $^{15}\text{N}$ residues (100N)	$^{14}\text{NH}_4\text{NO}_3$
3 Soil + $^{15}\text{N}$ residues (200N)	$^{14}\text{NH}_4\text{NO}_3$
4 Soil + $^{15}\text{N}$ residues (300N)	$^{14}\text{NH}_4\text{NO}_3$
5 Soil + $^{14}\text{N}$ residues (100N)	$^{15}\text{NH}_4\text{NO}_3$
6 Soil + $^{14}\text{N}$ residues (200N)	$^{15}\text{NH}_4\text{NO}_3$
7 Soil + $^{14}\text{N}$ residues (300N)	$^{15}\text{NH}_4\text{NO}_3$
8 Soil only (0N)	$\text{NH}_4^{15}\text{NO}_3$

TABLE II: AVERAGE GROSS MINERALIZATION RATE (STANDARD DEVIATION) AND PROPORTION OF GROSS MINERALIZATION FROM RESIDUES ( $\alpha$ )

Treatment	Injection	GMR ( $\mu\text{g N g}^{-1} \text{ day}^{-1}$ )	$\alpha$ (%)
Only soil	1	0.690(0.030)	
	2	1.49(0.076)	
	3	1.99(0.107)	
100N	1	0.808(0.303)	20.0(5.632)
	2	1.54(0.364)	6.47(0.551)
	3	1.54(0.530)	9.31(3.001)
200N	1	1.15(0.150)	34.5(2.938)
	2	2.30(0.200)	14.2(0.269)
	3	1.42(0.105)	17.8(1.128)
300N	1	1.62(0.344)	39.0(5.435)
	2	1.96(0.059)	24.4(1.734)
	3	1.21(0.268)	32.6(3.137)

FIG. 1. Nitrogen derived from residue (*Ndfres*) and soil (*Ndfsoil*).

**Conclusions.** The  $\alpha$  values increased with increasing residue addition, as expected. Temporal changes in GMR were detectable and showed that large additions of residue may lead to initial flushes of GM followed by reduction in GM of soil organic matter.

## REFERENCES

- [1] BROOKS, P.D., et al., Diffusion method to prepare soil extracts for automated nitrogen-15 analysis, *Soil Sci. Soc. Am. J.* **53** (1989) 1707–1711.
- [2] BARRACLOUGH, D., et al., The use of mean pool abundances to interpret  $^{15}\text{N}$  tracer experiments. I. Theory, *Plant Soil* **131** (1991) 89–96.
- [3] WATKINS, N., BARRACLOUGH, D., Gross rates of N mineralization associated with the decomposition of plant residues, *Soil Biol. Biochem.* **28** (1996) 169–175.



## **DIVINER 2000®: A NEW PORTABLE HAND-HELD DEPTH-RECOGNIZING SOIL-MOISTURE CAPACITANCE PROBE**

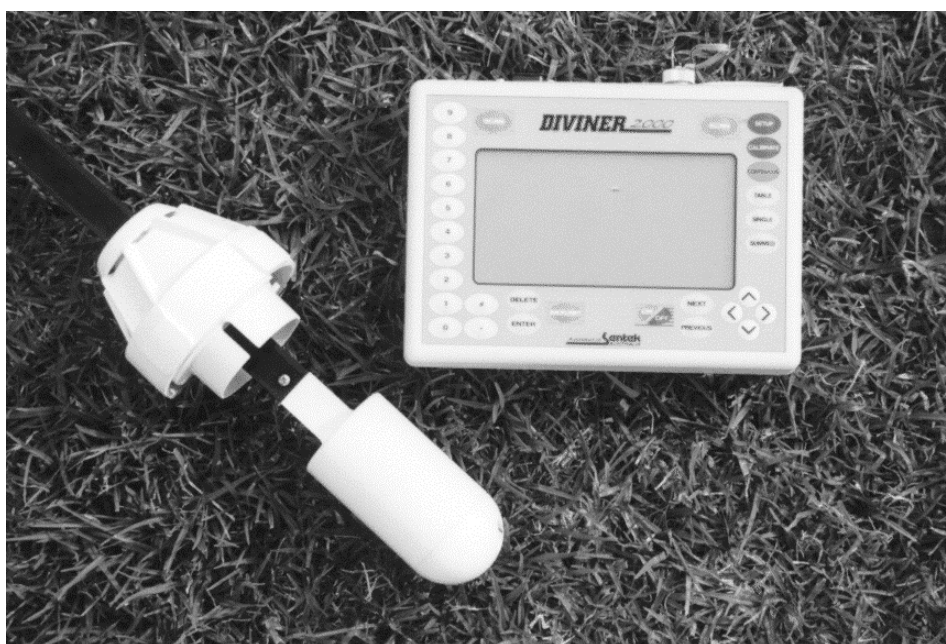
P. BUSS, A. FARES, M. DALTON  
Sentek Pty Ltd,  
Adelaide, Australia

The performance of a new portable hand-held capacitance probe capable of conducting very rapid multi-depth measurements of soil-water content was investigated and compared to the neutron thermalization method and other means of monitoring soil-water content. The Diviner 2000® consists of a hand-held, portable data logger with a liquid crystal display screen, connected by a cable to a depth-scaling probe rod with an attached capacitance sensor (Fig. 1).

The portable probe measures soil-water content at preset depth intervals of 10 cm through the soil profile to a maximum depth of 1.6 m, by swiping the sensor head down and up, without stopping, within a PVC access tube installed in the soil. The sphere of influence of the sensor penetrates the wall of the access tube to record soil-water content within 2 to 3 seconds. Soil-water profile data can be collected from an array of up to ninety-nine access tubes at selected sites.

Initial investigations of the core-sensor technology [1] using stationary (non-mobile sensors) yielded a regression of volumetric soil-water content on SF (scaled frequency = normalized sensor counts) for a Mattapex silt loam soil resulting in a highly significant ( $r^2 = 0.992$ , RMSE =  $.009 \text{ cm}^3 \text{ cm}^{-3}$ ,  $n = 15$ ), nonlinear relationship  $\theta_v = 0.490\text{SF}^{2.1674}$  (Fig. 2).

Instrument calibration of Diviner 2000® and the CPN 503 DR Hydroprobe® using a sand and a clay loam yielded highly significant relationships between the soil volumetric water content and instrument reading. These calibration results were achieved using the Diviner's ability to automatically take a reading in the soil every 10 cm over a depth of 1 m within 2.5 seconds, as compared to measurement of the same profile with the neutron probe in more than 1 min (4 to 16 seconds measurement time per depth level).



*FIG. 1. The Diviner 2000® portable hand-held depth-recognizing capacitance soil-moisture probe; left: probe rod with sensor head; right: hand-held data-display unit.*

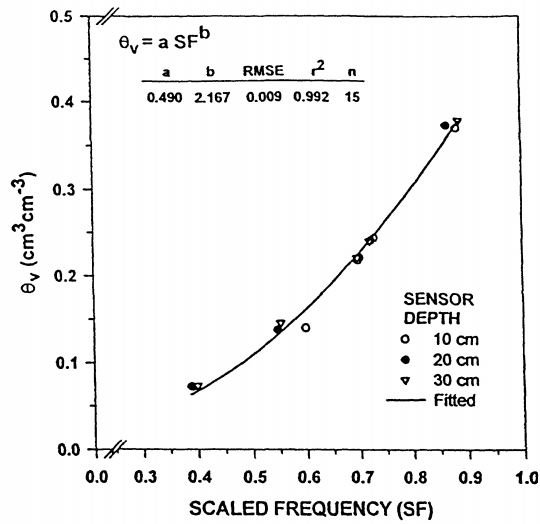


FIG. 2. Volumetric soil water content ( $\theta_v$ ) vs. scaled frequency (SF) on a Mattapex silt loam soil at Beltsville, MD. The regression relationship is represented as a solid line.

## REFERENCES

- [1] FARES, A., ALVA, A.K., Estimation of citrus evapotranspiration by soil water mass balance, *Soil Sci.* **164** (1999) 302–310.
- [2] PALTINEANU, I.C., STARR, J.L., Real-time soil water dynamics using multisensor capacitance probes: laboratory calibration, *Soil Sci. Soc. Am J.* **61** (1997) 1576–1585.

# EFFECTS OF PERENNIAL PASTURE SPECIES ON CLOVER N FIXATION ASSESSED BY THE $^{15}\text{N}$ -DILUTION METHOD

J. EVANS, B.S. DEAR, G. SANDRAL  
NSW Agriculture, Wagga Wagga

M.B. PEOPLES  
CSIRO, Canberra

Australia

Pasture production in Australia is traditionally based on annual legume and grass species that mature in early summer and regenerate from seed in mid to late autumn. In the absence of plants in summer and autumn, nitrate may accumulate and subsequently leach, acidifying the soil [1]. Over time, this process has resulted in extensively degraded pastures. Summer-active perennial species can reduce the rate of acidification occurring by this process by removing soil nitrate and water [2,3], however their impact on N fixation by the legume in the pasture in different environments is unknown.

Trials were established in 1995 at two sites with average annual rainfall: Junee (534 mm) and Ardlethan (445 mm). Subterranean clover was sown alone and with each of two perennial grasses, *Phalaris* sp. and *Danthonia* sp. The grasses were sown to give initial densities of 7.5, 15, 30, 60, and 120 plants/m<sup>2</sup>. (Note: *Eragrostis* also invaded plots at Ardlethan.) The effect of the perennial species and their density on clover N fixation was measured over a period of 4 weeks during spring of the following year, 1996. The percentage of clover N derived from fixation (*Pfix*) was measured using the  $^{15}\text{N}$ -dilution method [3], involving soil enrichment with  $^{15}\text{N}$ -labelled  $\text{KNO}_3$  and ryegrass as the reference plant, and converted to amounts of fixed N using clover dry matter and its N concentration.

At Junee, *Pfix* varied between 75 and 80% over most treatments, but reached 94% with the highest density of *Danthonia*. There was little effect of *Danthonia* on amounts of N fixed (Fig. 1) up to 40 plants/m<sup>2</sup>. *Phalaris* had a significantly ( $P < 0.05$ ) greater effect in reducing fixed N particularly at densities exceeding 30 plants/m<sup>2</sup>. Variation in fixed N was largely accounted for by the variation in clover dry matter ( $R^2 = 0.90$ ) that resulted from the grass treatment effects. At Ardlethan, *Pfix* ranged from 60 to 88%, being generally greater in clover associated with the grasses; however, much less N was fixed than at Junee. At similar plant densities, the grasses had a greater relative effect on fixed N than at Junee. Fixed N was markedly reduced by *Phalaris* at all densities, and by *Danthonia-Eragrostis* at densities  $>25$  plants/m<sup>2</sup> (Fig. 2). Variation in fixed N was primarily due to variation in clover dry matter ( $R^2 = 0.89$ ), but the reduction in fixed N caused by *Phalaris* also involved a significant reduction in the total N concentration in clover.

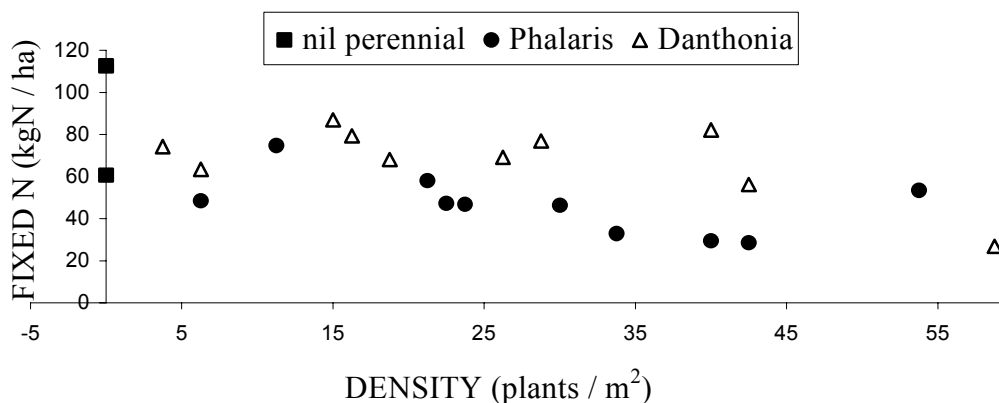


FIG. 1. Effect of perennial species and density on the quantity of N fixed by subterranean clover at Junee.

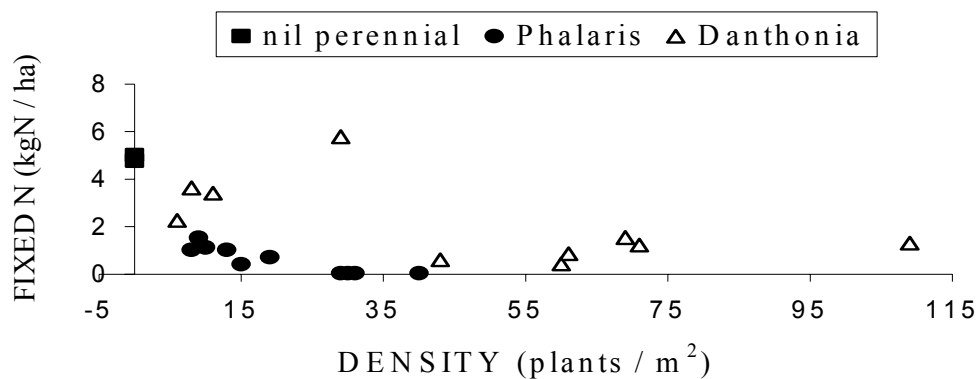


FIG. 2. Effect of perennial species and density on the quantity of N fixed by subterranean clover at Ardlethan.

This work is providing information on the more suitable perennial species to coexist with subterranean clover. In the drier zones, *Phalaris* is not suitable, and *Danthonia* is suitable only at low densities. With increasing rainfall, higher densities of *Danthonia* may be used, or low densities of *Phalaris*. The inclusion of grasses, nevertheless, represents a compromise between maximal N fixation and a more sustainable pasture with a more-even distribution of forage throughout the year.

#### REFERENCES

- [1] SIMPSON, J.R., Mineral nitrogen fluctuations in soils under improved pasture in southern New South Wales, Aust. J. Agric. Res. **13** (1962) 1059–1072.
- [2] RIDLEY, A.M., et al., Nitrate leaching under *Phalaris*, cocksfoot and annual ryegrass pastures and implications for soil acidification, Aust. J. Agric. Res. **50** (1999) 55–63.
- [3] CHALK, P.M. Estimation of N<sub>2</sub> fixation by isotope dilution: an appraisal of techniques involving <sup>15</sup>N enrichment and their application, Soil Biol. Biochem. **17** (1985) 389–410.

# IMPORTANCE OF DROUGHT STRESS AND NITROGEN FIXATION IN THE DESERT LEGUME *Alhagi sparsifolia* — RESULTS FROM $^{13}\text{C}$ AND $^{15}\text{N}$ NATURAL-ABUNDANCE STUDIES IN THE FIELD

S.K. ARNDT, A. KAHMEN, C. ARAMPATIS, M. POPP  
University of Vienna, Vienna, Austria

The perennial legume shrub *Alhagi sparsifolia* is a predominant member of the vegetation at a river oasis in the Chinese Taklamakan desert. As a wind-break, it helps prevent sand-dune movement, and it is the most important source of fresh and storage fodder for livestock. Although *Alhagi* displays potential for regeneration after harvest, its stands have been depleted through excessive exploitation and heavy grazing, and recommendations for sustainable growth and use are needed [1].

Ecological adaptation of plant species to arid environments is poorly understood. Water and nitrogen are likely to be the two major constraints to growth and production in the Taklamakan desert (35 mm annual precipitation) [2]. Plants must have special adaptations to avoid lethal water deficits. Moreover, the supply of inorganic nitrogen sources, e.g. nitrate and ammonium, may be restricted due to diminished mineralization. Therefore, as a legume, nitrogen fixation may play an important role in the nutrition of *A. sparsifolia*. To be able to make recommendations for sustainable use of *Alhagi*, a study on natural abundance of the stable isotopes,  $^{13}\text{C}$  and  $^{15}\text{N}$ , was conducted in the foreland of Qira oasis at the southern rim of the Taklamakan desert.

*Alhagi* bushes were sampled monthly during 1999, and carbon-isotope composition of leaves and leaf solutes were investigated as measures of long-term and short-term water restriction, respectively. Preliminary investigations in 1998 of *Alhagi* plants led to the assumption that individuals growing near the fields of the oasis assimilated inorganic nitrogen forms such as  $\text{NO}_3^-$  or  $\text{NH}_4^+$  ( $\delta^{15}\text{N}$  values of 5 to 8), whereas individuals growing close to the desert used  $\text{N}_2$  fixation as their main source of nitrogen ( $\delta^{15}\text{N}$  values near zero). Therefore, *Alhagi* plants were sampled along a gradient from the oasis into the desert.

The carbon-isotope data revealed that all *Alhagi* species were well supplied with water throughout the season. The  $\delta^{13}\text{C}$  values of leaves and solutes were consistently negative, indicating no long- or short-term drought stress at any time, and this was supported by other water-relations data. Thus, *Alhagi* plants seem to have contact with groundwater and an efficient water-conducting system; moisture deficiency was not a limiting factor.

The  $\delta^{15}\text{N}$  values of *Alhagi* leaves along a 5-km gradient from the Qira Research Station into the desert showed no significant trend (Fig. 1.). Some plants were clearly fixing atmospheric  $\text{N}_2$ , but most were non-fixing, and the overall pattern had a clustered character. The factors controlling  $\text{N}_2$  fixation of *Alhagi* are unknown.

Data from xylem-sap investigations (not shown) indicate high concentrations of  $\text{NO}_3^-$  in the transpiration stream, and assays of belowground water resources have shown high  $\text{NO}_3^-$  concentrations (15–30 mg/L). *Alhagi* plants with  $\delta^{15}\text{N}$  values close to zero in their leaves had lower concentrations of  $\text{NO}_3^-$  in the xylem sap.

However, small error bars of the measured  $\delta^{15}\text{N}$  values in Fig. 1 indicate that all plants at one sampling site had the same N-utilization strategy. Investigation of root systems provided an explanation for this pattern. An excavation to a depth of 3 m in August 1999 revealed that five *Alhagi* bushes shared a single root system and, thus, were clones.

It was also observed that *Alhagi* plants showed the ability to regenerate both from lateral- and from tap-root suckers.

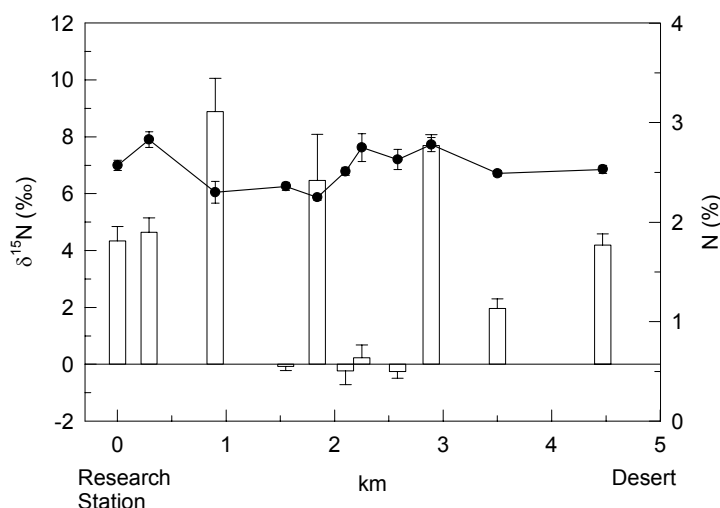


FIG. 1.  $\delta^{15}\text{N}$  values (bars) and nitrogen content (line) of *Alhagi sparsifolia* leaves along a desert gradient at Qira Research Station, China.

It is likely that *Alhagi* produces many of these clones [3]—some of which are  $\text{N}_2$  fixing and some of which are not. Notwithstanding this, the vegetative reproduction appears to be an important adaptation to an environment in which establishment of young plants is probably the major factor limiting propagation, and it explains why *Alhagi* grows in large stands in the oasis foreland.

However, whether an *Alhagi* plant was fixing  $\text{N}_2$  or not had no effect on the nitrogen content. All investigated plants displayed high content in their leaves (2–3% nitrogen) irrespective of the source (Fig. 1).

This investigation proved that neither water nor nitrogen limited growth of *A. sparsifolia*. Nitrogen fixation played only a minor role in most *Alhagi* individuals; therefore it was not a beneficial adaptation in this ecosystem. For re-establishment of *Alhagi* in the foreland, it appears that access to soil moisture is crucial until contact is made with groundwater sources. But, further studies are needed to prove this assumption, with emphasis on prerequisites for establishment at this site; very few seedlings of *A. sparsifolia* have been found in the oasis foreland.

The authors thank the European Commission/BBSRC for funding (contract ERB IC18-CT98-0275).

## REFERENCES

- [1] THOMAS, F.M., et al., Ecological basis for a sustainable management of the indigenous vegetation in a Central-Asian desert: presentation and first results, *J. Appl. Bot.—Angewandte Botanik* **74** (2000) 212–219.
- [2] XIA, X., et al., Desertification and Control of Blown Sand Disasters in Xinjiang, Science Press, Beijing (1993).
- [3] KERR, H.D., et al., Characteristics and control of camelthorn, *Weeds* **13** (1963) 156–163.

## A NEW APPROACH TO NITROGEN NUTRITION OF ROBUSTA COFFEE IN CÔTE D'IVOIRE\*

K. NGORAN, N.J. NGUESSAN, A. KONAN, G. YORO  
Centre National de Recherche Agronomique,  
Divo, Côte d'Ivoire

It has been reported that inorganic fertilizer at 100 kg N ha<sup>-1</sup>, on fairly unsaturated ferrallitic soils, gave coffee yields of 40% more than the untreated control. However, because fertilizers are too costly for small-scale farmers, this practice has not been widely adopted. On the other hand, studies have shown that legume trees contribute to soil improvement [1–4].

Therefore, a study was carried out in Divo, Côte d'Ivoire, to initiate the development of an agroforestry system involving coffee in association with legume trees.

The trial was conducted using a randomized block design with six treatments and five replicates. Coffee was intercropped with two nitrogen-fixing trees, *Gliricidia sepium* and *Albizia guachepele*, with the objective of improving the nitrogen nutrition of the coffee trees and minimizing need for fertilizer application. The treatments to the coffee trees were as follows:

- T1 = without legume trees and fertilizer (control),
- T2 = with *G. sepium*, without fertilizer,
- T3 = with *A. guachepele*, without fertilizer,
- T4 = with urea at the rate of 100 kg N ha<sup>-1</sup>,
- T5 = with *G. sepium*, with urea at 50 kg N ha<sup>-1</sup>, and
- T6 = with *A. guachepele*, with urea at 50 kg N ha<sup>-1</sup>.

The legume trees were planted in the coffee interrows, each at a density of 1,333 trees ha<sup>-1</sup> with a spacing of 3×2.5 m. Annual rainfall in Divo is about 1,400 mm and is bimodal (March–June and September–November) with two dry seasons (July–August and December–February).

Physical and chemical characteristics of the soil were as follows: clay + fine loam=28%; C%=1.19; N%=0.12; K meq/100=0.30; Ca meq/100=3.20; Mg meq/100=0.86; pH (H<sub>2</sub>O)=5.9.

One year after planting, the legume trees were cut at 1.5 m above ground. Every 3 to 4 months thereafter, they were pruned and the prunings were used as mulch around the coffee trees.

**Biomass of legume trees.** From 1996 to 1999, the biomass produced by *G. sepium* was 1.3 times higher than that produced by *A. guachepele*. During this period, *G. sepium* and *A. guachepele* supplied, respectively, the equivalent of 180 and 140 N ha<sup>-1</sup> year<sup>-1</sup> on average.

**Effect on coffee growth and yield.** During the first 2 years, measurements of coffee-tree growth parameters showed that the untreated controls were significantly ( $P < 0.05$ ) shorter than those in the other treatments. The number of fruiting nodes was significantly higher in the urea treatment than in all the others. The average yields from 1997 to 1999 confirmed the superiority of the urea treatment (Fig. 1).

Mulching stimulated growth of the coffee trees and gave yield increases of about 850 kg ha<sup>-1</sup> more than the untreated control; however, these differences were not significant. The results indicate that the use of legume-tree prunings to mulch coffee trees could help to minimize fertilizer application.

---

\*Work performed within the framework of IAEA projects IVC/5022 and IVC/5025.

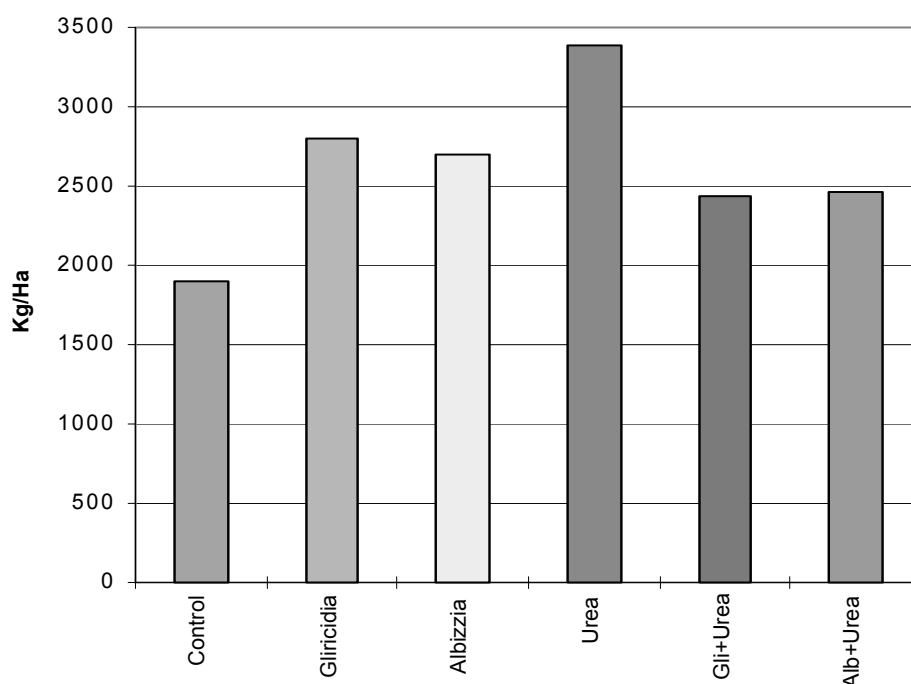


FIG. 1. Yields of coffee in response to urea and legume-tree prunings 1997–1999.

#### REFERENCES

- [1] BOUHARMONT, P., L'utilisation des plantes de couverture et du paillage dans la culture du caféier Arabica au Cameroun, *Café Cacao Thé* **23** (1979) 75–102.
- [2] BORNEMISZA, E., Nitrogen cycling in coffee plantations, *Plant Soil* **67** (1982) 241–246.
- [3] SNOECK, D., Interaction Entre Végétaux Fixateurs d'Azote et Non Fixateurs en Culture Mixte: Cas des *Leucaena* spp. Associées à *Coffea arabica* L. au Burundi, Thèse de Doctorat, Université Claude Bernard, Lyon (1995).
- [4] BEER, J., et al., Shade management in coffee and cocoa plantations, *Agroforestry Systems* **38** (1998) 139–164.



## NITROGEN RECYCLING IN A POTATO- MAIZE-POTATO SEQUENCE

G. DUEÑAS, O. MUÑIZ

Soil Institute,  
Havana, Cuba

T. LOPEZ

National Science Directorate,  
Havana, Cuba

F. ZAPATA

International Atomic Energy Agency,  
Vienna, Austria

In a series of pot and field experiments,  $^{15}\text{N}$  was used to investigate N recycling, and to validate crop-production conditions, in a potato-maize-potato rotation.

The results showed that a considerable amount of N, residual from the first potato crop, was available to the maize. This N, accumulated in the system, can be used by the maize, and thus minimize the N applied to this crop [2].

The application of  $100 \text{ kg N ha}^{-1}$  to the maize, together with the N applied to the previous crop, decreased the fertilizer utilization and increased the concentration of nitrate in the water. In such conditions, the yield of the maize crop did not justify application N at levels over  $30 \text{ kg N ha}^{-1}$ . The influence of the management of the previous crop is shown in Table II; it produced greater availability of N (which was not taken in consideration for the fertilization of potato), leading to a diminution in uptake of directly applied N by the potato crop, and to augmentation of nitrate concentration in the tubers.

These results were corroborated in maize-potato production fields in seven farms in Havana province. Table III shows that the fields in which the previous crop of maize had been fertilized at  $100 \text{ kg N ha}^{-1}$  had higher levels of N availability, resulting in higher nitrate concentrations in the tubers, but with lower tuber yields.

TABLE I. RESULTS OBTAINED BY THE ISOTOPE METHOD, MAIZE

Corn fertilization	N yield ( $\text{kg N ha}^{-1}$ )	Ndff (%)	N fert. yield ( $\text{kg N ha}^{-1}$ )	N fert. utilization (%)
$30 \text{ kg N ha}^{-1a}$	75.4	15	11.3	37.8
$100 \text{ kg N ha}^{-1a}$	85.8	8.5	7.29	7.29

Fertilizer applied to potato =  $180 \text{ kg N ha}^{-1}$ . <sup>a</sup>Urea at 10%  $^{15}\text{N}$ .

TABLE II. RESULTS OBTAINED BY THE ISOTOPE METHOD, POTATO (SECOND YEAR)

Fertilizer to the previous maize	N-fertilizer utilization <sup>a</sup> (%)	Nitrate in tubers ( $\text{mg kg}^{-1}$ )
$30 \text{ kg N ha}^{-1b}$	44	150
$100 \text{ kg N ha}^{-1b}$	35	250

<sup>a</sup>Fraction of the  $180 \text{ kg N ha}^{-1}$  applied to potato. <sup>b</sup>Urea at 10%  $^{15}\text{N}$ .

TABLE III. POTATO-YIELD DATA

Farm	Field	Previous corn fertilization (kg N ha <sup>-1</sup> )	Soil N availability (kg N ha <sup>-1</sup> )	Yield (t ha <sup>-1</sup> )	Tuber nitrate (mg N kg <sup>-1</sup> )
Batabanó	C-1	100	139	15.8	325
Melena	C-4	100	145	15.8	350
19 Abril	C-23	30	53.2	19.4	185
Güira	C-24	30	89.6	23.0	210
Güines	C-23	30	57.0	19.4	177

These results suggest the need to reduce N-fertilizer application to maize, which would also reduce the nitrate pollution of the environment.

This work was carried out with the collaboration of the International Atomic Energy Agency through the project ARCAL XXII.

#### REFERENCES

- [1] ANONYMOUS, Use of Nuclear Techniques in Studies of Soil-Plant Relationships, Training Course Series No. 2, International Atomic Energy Agency, Vienna (1990).
- [2] ZAPATA F., HERA C., "Enhancing nutrient management through use of isotope techniques", Nuclear Techniques in Soil Studies for Sustainable Agriculture and Environmental Preservation, IAEA, Vienna (1995) 83–105.

## MODIFICATION OF PHOSPHATE-ION TRANSFER IN A CROPPED COLOMBIAN OXISOL WITH P FERTILIZATION\*

C. MOREL

INRA-Agronomie,  
Villenave d'Ornon Cedex, France

P.G. SABO

IRI,  
Niamey, Niger

D. FRIESEN

CIMMYT,  
Nairobi, Kenya

The transfer of P ions between soil and solution is a factor that often limits plant production in Oxisols in native savannahs of Colombia. Changes in this transfer due to P fertilization and cropping were examined by isotope dilution. For a given P addition, cropping and fertilization significantly decreased the affinity of the soil's solid phase to react with P ions in solution, giving a higher concentration of P ions in solution and replenishment of P in cropped soils.

**Introduction.** The ability of the soil solid phase to react with phosphate (P) ions in solution is of prime importance to characterizing soil P availability and P fertilizer efficiency, especially in P-deficient Oxisols. Changes in P-ion transfer between the liquid and soil solid phases were analyzed in a cropped and P-fertilized Colombian Oxisol by isotope dilution.

**Material and methods.** Soil samples were taken in 1997 from a long-term field experiment established in 1991 at the ICA-CIAT, Carimagua Research Station (Meta, Colombia). The experiment had a completely randomized block design, with sixteen treatments in four replications. Only the three following treatments are reported here: i) unfertilized (T1); ii) 50 kg P as TSP ha<sup>-1</sup> applied every year from 1991 to 1995 (T11); iii) 200 kg P ha<sup>-1</sup> soluble phosphate fertilizer (TSP) applied once only at the beginning of the experiment (T16).

The soil is an Oxisol (tropeptic haplustox, isohyperthermic) with a silty clay loam texture. All soil samples were air-dried and 2-mm sieved before P determination. All soil samples were P-enriched by adding 40, 100, 150, 200, 250, 300 mg P kg<sup>-1</sup> as KH<sub>2</sub>PO<sub>4</sub> in soil suspensions (1 g:10 mL) to obtain, after 40 h of incubation, soil-solution P ( $C_p$ ) ranging from about 0.05 to 5 mg P L<sup>-1</sup>. In all P-enriched soil samples, isotopically exchanged P ( $E$ ) was determined by labelling P ions in solution with <sup>32</sup>PO<sub>4</sub> and applying the isotope-dilution principle, i.e. the isotopic compositions of P ions in solution equals the isotopic composition of  $E$  [1]:

$$R/E = r/Q_w \quad (1)$$

where  $R$  is <sup>32</sup>PO<sub>4</sub> introduced in solution at  $t=0$ ,  $r$  is that remaining in solution after time  $t$ , and  $Q_w$  the amount of P ions in solution, i.e.  $C_p$  multiplied by the volume-to-mass ratio.

**Results and discussion.** Figure 1 describes  $C_p$  values as a function of added P for all treatments and blocks. For all laboratory P additions,  $C_p$  values of the T1 treatment were significantly smaller than those of T11 and T16. For instance,  $(T11-T1)/T1$  was about 50% for the 40 mg P kg<sup>-1</sup> addition. As a consequence, added P sorbed onto the soil's solid phase was significantly greater for the T1 treatment.

The reactivity the solid phase in T1 decreased after P fertilization. This decrease is partly explained by the increase in  $C_p$ , which reduced reactions of P ions in solution with the solid phase, possibly attributable to modifications in the intrinsic properties of transfer of P ions between solution and soil. This change was analyzed by determining  $E$  as a function of time and  $C_p$  (Fig. 1):

---

\*This work received the support of fellowship C6/NER/98012R from IAEA.

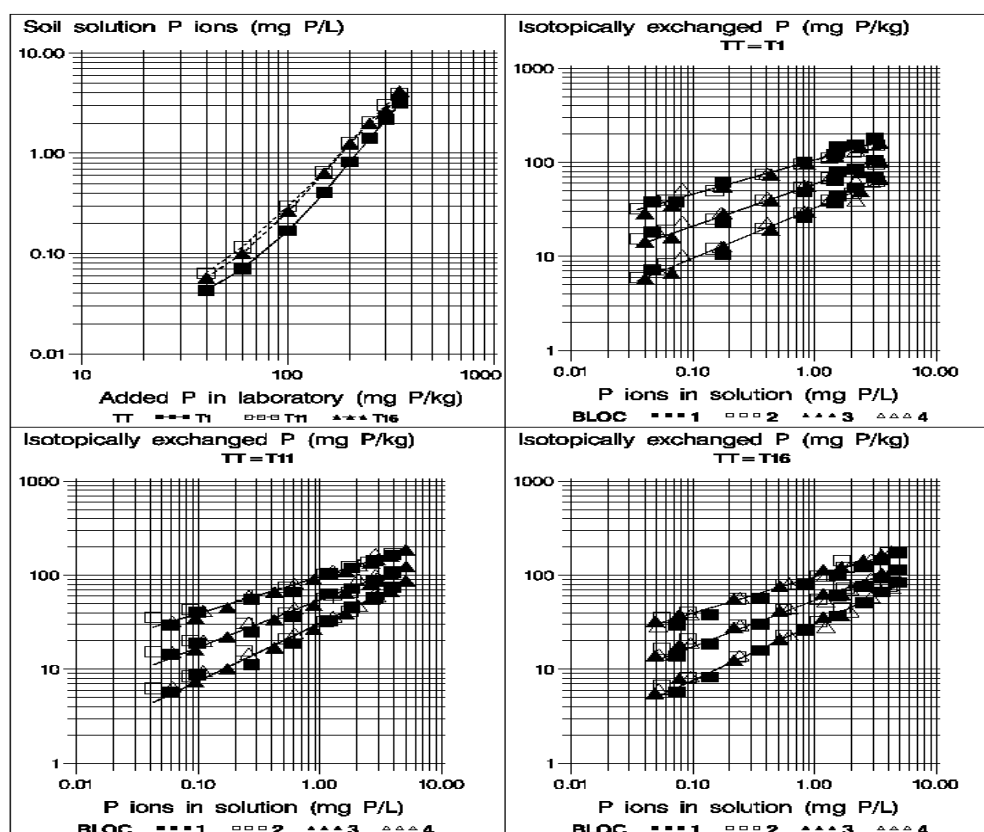


FIG. 1. Upper left: soil solution P ( $C_p$ ) vs. added P, unfertilized (T1) or fertilized with 50 kg P ha<sup>-1</sup> yr<sup>-1</sup> for 5 years (T11) or fertilized once with 200 kg P ha<sup>-1</sup> at the start (T16). Upper right and below: isotopically exchangeable P as a function of time and soil-solution P ions for T1, T11 and T16. For a given soil, the different superimposed data set corresponds to 1, 10 and 100 min of isotope exchange. [Symbols: experimental data. Lines: equations (2), (3) and (4) of the text.]

$$\text{For T1: } E = 32.9 \times C_p^{0.53} t^{0.255 - 0.037 \text{LN}(C_p)}, 24 \text{ obs.}, R^2 = 0.997 \quad (2)$$

$$\text{For T11: } E = 30.6 \times C_p^{0.62} t^{0.249 - 0.048 \text{LN}(C_p)}, 24 \text{ obs.}, R^2 = 0.998 \quad (3)$$

$$\text{For T16: } E = 29.8 \times C_p^{0.61} t^{0.253 - 0.045 \text{LN}(C_p)}, 24 \text{ obs.}, R^2 = 0.998 \quad (4)$$

The  $E$  description for T1 was significantly different from the two others, whereas there were no significant differences for T11 and T16 data. Differences in  $E$  values between fertilized and unfertilized treatments changed with time and  $C_p$ . At 0.001 mg P L<sup>-1</sup>, the  $E$  values calculated with T1 were 0.8, 2.7, 8.6, and 33.5 mg P kg<sup>-1</sup> after 1, 10, 100, and 1,440 min, respectively. Using the T11 description, these  $E$  values were 0.4, 1.6, 6.0, and 28.5 mg P kg<sup>-1</sup> showing a relative (T11-T1)/T1 decrease in P transfer of between -50% after 1 min to -15% after 1 day. As already observed in grass-legume pastures [2], this decrease likely resulted from the greater content of organic carbon in fertilized soils due to increases in root biomass and return of leaves and stems. Organic compounds competing with P ions for reactions sites reduce soil-P sorption.

In conclusion, cultivation and fertilization of one savannah Oxisol significantly decreased the affinity of the soil's solid phase to react with P ions in solution.

## REFERENCES

- [1] MOREL, C., et al., Transfer of P ions between soil and solution: perspectives in soil nutrient testing, J. Environ. Qual. **29** (2000) 50–59.
- [2] OBERSON, A., et al., 1999. Phosphorus status and cycling in native savanna and improved pastures on an acid low-P Colombian Oxisol, Nut. Cycling Agroecosystems **55** (1999) 77–88.

# DETERMINATION OF URANIUM UPTAKE BY PLANTS BY MEANS OF INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

J. FLECKENSTEIN, E. SCHNUG

Institute of Plant Nutrition and Soil Science,  
Braunschweig, Germany

M.C. MEYER, T. McLENDON

Shepherd Miller, Inc.,  
Fort Collins, Colorado, United States of America

D. PRICE

United States Army Construction Engineering Research Laboratory,  
Champaign, Illinois, United States of America

**Introduction.** Uranium contamination of the environment may occur naturally, but can also occur from the use of phosphorus fertilizers, or in military areas from deployment of depleted uranium rounds. Depleted uranium contamination can occur as a result of testing and training of munitions, as well as “collateral damage” from use of uranium rounds in combat. Though the radioactive hazards are slight, depleted uranium can have toxic effects on biochemical processes. The aim of the presented study was to monitor the bio-availability of uranium (in this particular case as depleted uranium derived from a military proving ground) for three species of grass.

**Materials and methods.** A pot experiment was carried out by Meyer et al. [1] with *Aristida purpurea*, *Buchloe dactyloides*, and *Schizachyrium scoparium*.

TABLE I. ABOVE-GROUND PLANT-TISSUE CONCENTRATION OF DEPLETED URANIUM (DRY WEIGHT BASIS) FOR THREE SPECIES, THREE WATER LEVELS, AND FIVE URANIUM TREATMENTS

Water regime/Species		Depleted uranium applied at (mg kg <sup>-1</sup> )					Average
		0	10.5	105	1,050	5,250	
		(mg kg <sup>-1</sup> )					
Low	<i>A. purpurea</i>	4.7	3.0	8.9	6.8	34.0	22.4
	<i>B. dactyloides</i>	2.2	3.3	4.7	60.6	57.0	
	<i>S. scoparium</i>	1.9	2.0	9.8	12.1	125	
Moderate	<i>A. purpurea</i>	4.1	3.0	2.8	13.9	34.5	18.5
	<i>B. dactyloides</i>	1.5	2.8	7.8	31.3	81.3	
	<i>S. scoparium</i>	2.2	1.9	9.1	33.7	47.8	
High	<i>A. purpurea</i>	3.6	3.7	2.6	58.4	417	68.3 <sup>a</sup>
	<i>B. dactyloides</i>	3.9	5.0	6.5	36.2	188	
	<i>S. scoparium</i>	2.0	2.3	28.3	131	136	
Average		2.9	3.0	8.9	42.7	124 <sup>b</sup>	

<sup>a</sup>High water value is significantly different ( $P = 0.05$ ).

<sup>b</sup>5,250 mg kg<sup>-1</sup> value is significantly different ( $P < 0.0001$ ).

The growth substrate was a clean local sand to which depleted uranium (DU) from a military testing site was added. Ground DU material was mixed into the upper layer (6 cm of 36-cm deep pots) of the substrate with the following concentration: (mg/kg): 0, 50, 500, 5,000, 25,000. The resultant per-pot uranium concentrations were calculated as 0, 10.5, 105, 1,050, and 5,250 mg/kg.

Three water regimes were applied during the growth period of 82 days. The grass samples were harvested and separated as above-ground and below-ground materials. Yields of biomass were determined.

Digestion was performed on 50- to 100-mg samples in closed teflon vessels with 4 mL HNO<sub>3</sub> and 1 mL H<sub>2</sub>O<sub>2</sub> in a microwave furnace and afterwards brought to a volume of 10 mL with double-distilled water. The <sup>238</sup>U measurements were carried out by means of an inductively coupled plasma mass spectrometer (Plasmaquad 3, Unicam).

**Results and discussion.** In Table I, the results are summarized for the uranium concentrations in the above-ground tissues. Uranium was taken up by the plants at all concentration treatments. Accumulation in the roots was greater than in the above-ground portions. Expressed as concentration ratios [CR; (U in tissue)/(U in soil)], all of mean above-ground CRs for each treatment are less than 0.3. The mean root CR values, however, were as high as 2.3, indicating that uranium may be concentrated in root tissue. This suggests that uranium, as supplied as munition-based DU, is available to plants, and thus may enter the food chain as a potential hazard to humans and animals.

#### REFERENCE

- [1] MEYER, M.C., et al., Evidence of depleted uranium-induced hormesis and differential plant response in three grasses, *J. Plant Nutr.* **21** (1998) 2475–2484.

## UTILIZATION OF NITROGEN BY TWO RICE VARIETIES AT VARIOUS NPK LEVELS

M.B. ONCSIK

Irrigation Research Institute,  
Szarvas

F. KŐRÖSI

Szent István University,  
Gödöllő

Hungary

Our objective was to assess the efficiency of utilization of N by rice, as a basal application or as a top dressing or both, vs. genotype—an important and controversial issue [1]. Nitrogen-15 labelling was adopted [2].

The effects of genotype and N, P, and K levels, with basal and split N dressings, were investigated in pots, in terms of recovery of fertilizer  $^{15}\text{N}$  in the straw and grain. The following treatments were applied (ppm): 1,  $\text{N}_0\text{P}_0\text{K}_0$ ; 2,  $\text{N}_0\text{P}_{300}\text{K}_{300}$ ; 3,  $\text{N}_{300}\text{P}_{300}\text{K}_{300}$ ; 4,  $\text{N}_{600}\text{P}_{300}\text{K}_{300}$ ; 5,  $\text{N}_{900}\text{P}_{300}\text{K}_{900}$ ; 6,  $\text{N}_{600}\text{P}_{600}\text{K}_{900}$ ; 7,  $\text{N}_{900}\text{P}_{900}\text{K}_{900}$ ; 8,  $\text{N}_{300+300}\text{P}_{600}\text{K}_{900}$  (N top dressing applied at tillering); 9,  $\text{N}_{300+300+300}\text{P}_{900}\text{K}_{900}$  (N top dressing applied at tillering and panicle initiation).

To examine a genotype effect, cvs. Sandóra and Agusztá were investigated. The  $^{15}\text{N}$  uptake from  $[(\text{NH}_4)_2\text{SO}_4, 10 \text{ atom } \%]$  was measured using a Fisons NA 1500 C/N analyser coupled to a GC-MS (Fisons 8000 GC + MD800 quadruple MS) measuring unit. For data evaluation, mean values with standard errors (SE) were determined, and principal component analysis was applied.

Genotype $\times$ NPK interactions were revealed for  $^{15}\text{N}$  accumulation. The overall average for the nine treatments with Agusztá indicated a recovery of 74 mg N/g dry matter from the ammonium sulphate more than with Sandóra. For Agusztá, fertilizer-N uptake from the basal dressing of  $\text{N}_{300}\text{P}_{300}\text{K}_{300}$  (Treatment 3) was within the SE of that for  $\text{N}_{300+300}\text{P}_{600}\text{K}_{900}$  (Treatment 8) (Fig. 1). This did not hold for Sandóra (Fig. 2). From the point of view of basal dressing vs. split, it is important to note that, for both varieties, the  $^{15}\text{N}$ -uptake from  $\text{N}_{300+300+300}\text{P}_{900}\text{K}_{900}$  (Treatment 9) did not significantly surpass that from  $\text{N}_{600}\text{P}_{600}\text{K}_{900}$  (Treatment 6); in fact, in the case of Sandóra, the latter treatment was superior (cf. Figs. 1 and 2).

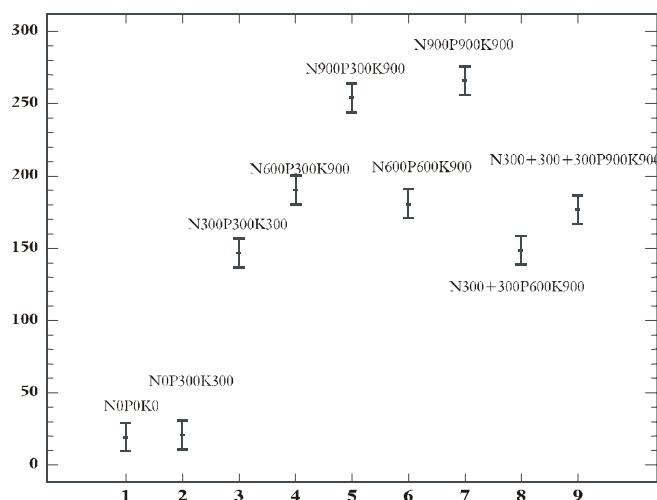


FIG. 1. Effect of treatments (1–9) on  $^{15}\text{N}$  recovery by rice cv. Agusztá (mg  $^{15}\text{N}$ /g DM).

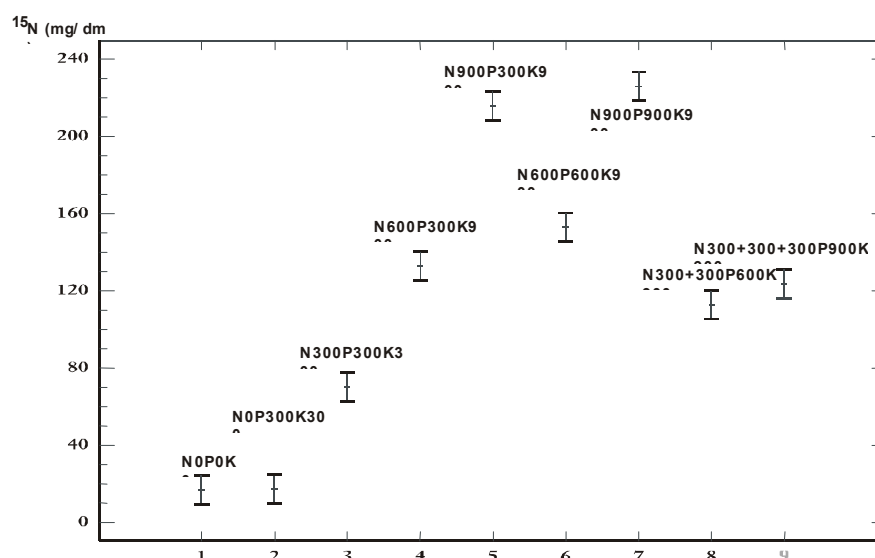


FIG. 2. Effect of treatments (1–9) on  $^{15}\text{N}$  recovery by rice cv. Sandóra. (mg  $^{15}\text{N}$ /g DM).

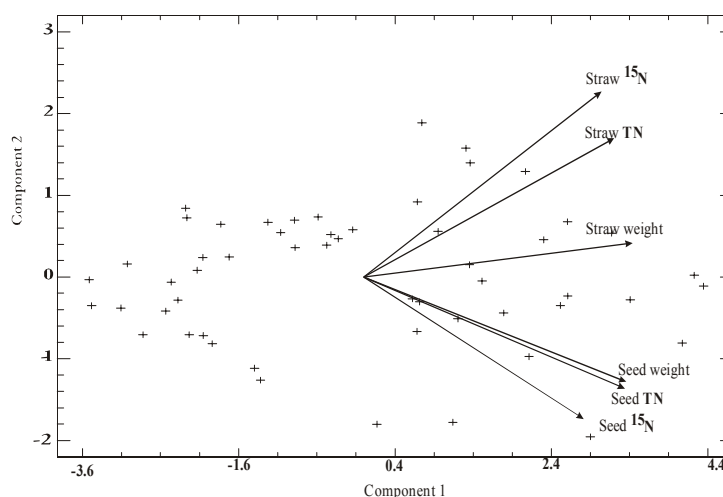


FIG. 3. Biplot demonstration of the connection between labelled N and N contents as well as seed and straw weights.

The highest incorporations of  $^{15}\text{N}$ , for both varieties, were obtained with  $\text{N}_{900}\text{P}_{300}\text{K}_{900}$  (Treatment 5) and  $\text{N}_{900}\text{P}_{900}\text{K}_{900}$  (Treatment 7). The biplot (component weights and eigen vectors) demonstration of principal component analysis in a six-dimensional sample space revealed a close connection between seed weight and total seed N-content (TN), with no connection with straw weight (Fig. 3).

In summary, top dressing was not as effective as basally applied N.

## REFERENCES

- [1] HIRANO, M., et al., Effects of combined practice of nitrogen application regime with sparse planting on growth and yield of rice, Japanese J. Crop Sci. **66** (1997) 551–558.
- [2] BUFOGLE, A., Jr., et al., Microplot size and retainer effects on rice growth and nitrogen-15 accumulation, Agron. J. **89** (1997) 567–571.



## EVALUATION AND MANAGEMENT OF MICRONUTRIENTS FOR OPTIMIZING RICE PRODUCTIVITY IN VALLEY SOILS

P. NONGKYNRIH, A.K. SINGH, G. DKHAR  
North-Eastern Hill University,  
Shillong, India

Rice is the major crop in Meghalaya, a hill state in northeastern India has rice. The altitude ranges from 80 m to 1,850 m above MSL, with an average annual rainfall of 2,000 to 3,000 mm. It is found that for top yields and profits, due importance to micronutrients should be given along with major plant nutrients.

A few micronutrients have been studied to determine their status in soils and the rice crops they support. Among them, Zn and Cu have been found to be of low content in soils, ultimately causing deficiencies in rice plants growing under submerged condition. Findings show that a major portion (~65%) of added Zn and Cu may be converted into relatively unavailable forms, and only small amounts remain readily available for uptake by plants. Soil properties—pH, organic carbon, CEC, and clay content—influence the availability of added Zn and Cu. In acid soils Zn and Cu can be 50 to 75% fixed when a solution containing these elements is applied.

The adsorption of Zn (labelled with  $^{65}\text{Zn}$ ) and its subsequent desorption in soils growing rice of varying pH, organic carbon, and cation-exchange capacity were studied in the laboratory. Five surface soil samples (0–20 cm) were collected from several rice-growing areas of Meghalaya State. Ten-g portions of air dried soil (<2 mm) in polypropylene centrifuge tubes were equilibrated with 20-mL aliquots of supporting electrolyte (0.01 M  $\text{CaCl}_2$ ) containing graded levels of Zn, 0, 2, 5, 10, 15, 20, and 25  $\text{mg mL}^{-1}$ , tagged with  $^{65}\text{Zn}$  (1.0 mCi  $\text{g}^{-1}$  Zn), at 25°C for 24 h. Each soil suspension was centrifuged, followed by filtration of the supernatant to remove particulates. The radioactivity of  $^{65}\text{Zn}$  was measured in the filtrate using a well-type solid scintillation counter (Nucleonix-GR611M). The amount of Zn adsorbed was computed from the difference in the initial and final radioactivities of  $^{65}\text{Zn}$  in the filtrate from each soil. To interpret the adsorption of Zn in soils, the Langmuir adsorption equation was employed:

$$C/x/m = 1/kb + C/b$$

where

C is the equilibrium concentration of Zn in soil solution ( $\mu\text{g mL}^{-1}$ ),  
x/m is the amount of Zn adsorbed ( $\mu\text{g g}^{-1}$  soil),  
b is the adsorption maxima ( $\mu\text{g g}^{-1}$  soil),  
and k is the bonding energy constant ( $\text{mL } \mu\text{g}^{-1}$ ).

Desorption of Zn was studied by shaking soil residues obtained from the adsorption study with 20 mL of supporting electrolyte (0.01 M  $\text{CaCl}_2$ ) solution for 18 h then centrifuging and filtering. This process was performed twice. The soil residues were subsequently shaken with 20 mL of complexing agent (1%  $\text{Na}_2\text{-EDTA}$ ) for 6 h and centrifuged and filtered. The remaining soil residues were extracted with 20 mL of 0.1 M HCl by shaking for 6 h. In each of the four extracts, radioactivity of  $^{65}\text{Zn}$  was measured in a well-type solid scintillation counter with an NaI(Tl) detector. All measurements were made in duplicate.

The data revealed that the amount of Zn adsorbed by different soils increased with concentration of added Zn. But, on average, percent adsorption of Zn greatly decreased, from 95 to 62%, with increasing concentration of Zn in the equilibrium solution. The value of adsorbed Zn also depended on physico-chemical properties of the soil; the value of adsorbed Zn increased as the pH of the soils increased. Regarding desorption of Zn by different extractants, the data indicated that, as the concentration of added Zn increased, the percent desorption of Zn also increased. At lower concentrations of added Zn, per cent Zn desorbed was much less as compared to that desorbed at higher concentrations.

From our experiment, we conclude that wetland rice soils of Meghalaya with DTPA-extractable Zn  $<1.2 \text{ mg kg}^{-1}$  and DTPA-extractable Cu  $<0.7 \text{ mg kg}^{-1}$  and rice plants with Zn concentrations of  $<35.9 \text{ mg kg}^{-1}$  and Cu concentrations of  $<7.0 \text{ mg kg}^{-1}$  will need Zn and Cu fertilization to meet yield requirements.

Positive effects of Zn and Cu application on rice yields have been obtained. Increases in paddy yield due to Zn application were up to 40%, and, due to Cu application, up to 29%. Hence, Zn and Cu deficiencies prevent high-yielding varieties from yielding optimally with NPK. Moreover, the results indicate that Zn at  $5.0 \text{ kg ha}^{-1}$  and Cu at  $2.5 \text{ kg ha}^{-1}$  are the most economical rates, and may be applied to the soil at the time of puddling.

## REFERENCES

- [1] SACHDEV, P., et al., Adsorption-desorption of radiocaesium in semi-arid and tropical soils, J. Nuclear. Agric. Biol 24 (1995) 201–209.
- [2] SWARUP, A., et al., Sorption and desorption of Zn, Pb and Cd by soil under forest, J. Indian. Soc. Soil Sci. **43** (1995) 38–42.

## EFFECT OF AGRICULTURAL COUNTERMEASURES ON THE TRANSFER FACTOR OF CAESIUM-137 IN ONION (*Allium cepa* L.)

P.V. JEGADEESWARI, M. SHANMUGAM, A.R. RAJAN  
Tamil Nadu Agricultural University,  
Coimbatore, India

A field experiment, with onion cv. Rampur as the test crop, was conducted to study the effect of two levels (3.7 and 7.4 MBq m<sup>-2</sup>) of <sup>137</sup>Cs and three countermeasures (application of farmyard manure at 12.5 t ha<sup>-1</sup>, application of extra potassium 100% in excess of that recommended for onion, and deep ploughing with a mold board plough). The crop was harvested at maturity and separated into bulb and sheath and analysed for <sup>137</sup>Cs activity using a NaI (TI) gamma ray spectrometer. The level of <sup>137</sup>Cs significantly influenced the transfer factor (TF) in bulbs and sheaths. The levels of <sup>137</sup>Cs and TF values were inversely related. The countermeasures had significantly influenced the TF of <sup>137</sup>Cs from soil to onion: the values ranged from 0.0009 to 0.0025 m<sup>2</sup> kg<sup>-1</sup>. The TF was highest for the control and the three countermeasures had significantly reduced values. Application of FYM was the most effective of the countermeasures. The other two countermeasures were on a par. They greatly reduced the TF also in onion sheaths; all three countermeasures had similar effects, and recorded significantly lower TF values than did the control.

**Introduction.** Caesium-137 has drawn attention because of its long half-life (30 years) and its threat to ecosystems and to the human food chain owing to its chemical similarity to K. Research is needed on the consequences, for agriculture and food production, of <sup>137</sup>Cs fallout from weapons testing and/or nuclear reactor accidents, in both the short- and long-term perspectives. Therefore, the present investigation was carried out with the objective of understanding the effects of <sup>137</sup>Cs contamination of soil and the effect of agricultural countermeasures on the transfer of <sup>137</sup>Cs from soil to onion crops under field conditions.

**Methodology.** The experiment was conducted at Tamil Nadu Agricultural University farms, Coimbatore, India, on a clay loam soil. Microplots (1×1 m) were delineated in the centre of larger plots (5×5 m) after field preparation. The microplots were contaminated with two levels of <sup>137</sup>Cs (3.7 and 7.4 MBq m<sup>-2</sup>) by uniformly applying a carrier-free solution of caesium nitrate with a hand sprayer. Then three treatments were imposed as countermeasures on the plots, viz., application of farm yard manure (FYM) at 12.5 tonnes ha<sup>-1</sup>, application of 100% extra dose of potassium over that recommended for onion, and deep ploughing with a mold board plough. Controls had no countermeasure.

Basal applications of nitrogen (50 kg N ha<sup>-1</sup> as urea) and phosphorus (50 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> as superphosphate) were made to all the plots. Potassium was applied at 100 kg K<sub>2</sub>O ha<sup>-1</sup> against the recommended dose of 50 kg K<sub>2</sub>O ha<sup>-1</sup> for onion, as muriate of potash. After imposing these treatments, onion variety Rampur was sown in the plots with a spacing of 45×10 cm. All treatments were replicated thrice in a randomized block design. The crop was harvested at maturity, separated into bulb and sheath, and analysed for <sup>137</sup>Cs activity using a NaI (TI) gamma ray spectrometer by differential counting. From the radioassay data, the transfer factor (TF) of <sup>137</sup>Cs was computed as follows:

$$TF (m^2 kg^{-1}) = \frac{{}^{137}\text{Cs activity in plant sample (MBq kg}^{-1}\text{)}}{{}^{137}\text{Cs activity deposited on soil (MBq kg}^{-1}\text{)}}$$

**Effects of treatments on onion bulbs.** The TF of <sup>137</sup>Cs from soil to onion bulb was found to be significantly influenced by the countermeasures; the values ranged from 0.0009 to 0.0025 m<sup>2</sup> kg<sup>-1</sup> (Table I). The TF was highest in the control and each of the countermeasures significantly reduced it. Application of FYM was the most effective. The other two, viz., potassium fertilization and deep ploughing produced similar TF values. Though TFs tended to decrease with the increase in the level of <sup>137</sup>Cs contamination, the decrease was not significant.

**Effects of treatments on onion sheath.** In contrast to bulbs, the level of  $^{137}\text{Cs}$  significantly affected TF values of the onion sheaths (Table II). The TFs and the  $^{137}\text{Cs}$  levels were inversely related, probably due to the reduction in molar fraction of Cs in the alkali metal pool with increasing addition to the soil. The countermeasures greatly reduced the TF values for the sheaths. All three had similar effects, providing significantly lower TF values than that of the control. The TF in the FYM plots was the lowest, attributable to adsorption of  $^{137}\text{Cs}$  by the organic matter [1,2]. It was evident that potassium application and deep ploughing are also effective measures for reducing  $^{137}\text{Cs}$  contamination. The antagonism between K and Cs is well known. Deep ploughing removes  $^{137}\text{Cs}$  from the root zone.

**Conclusions.** The TF constitutes the key to predicting amounts of radionuclides that may reach the human population through the food chain. In the event of a radioactive release of  $^{137}\text{Cs}$ , action may be directed towards limiting the dose caused by contamination of agricultural land through practices such as direct application of organic manures, potassium sources, and deep ploughing. In the present investigation all three countermeasures significantly reduced the TF in both the bulb and sheath of onion. The TF and  $^{137}\text{Cs}$  contamination levels appeared to be inversely related, though this relationship was significant only in the case of onion sheaths.

TABLE I. EFFECT OF LEVEL OF  $^{137}\text{Cs}$  AND COUNTERMEASURES ON SOIL-TO-CROP TF OF  $^{137}\text{Cs}$  ( $\text{m}^2 \text{kg}^{-1}$ ) IN ONION BULBS

Countermeasure	Level of $^{137}\text{Cs}$		Mean
	3.7 ( $\text{MBq m}^{-2}$ )	7.4 ( $\text{MBq m}^{-2}$ )	
None/control	0.0023	0.0027	0.0025a
FYM	0.0010	0.0008	0.0009c
K fertilizer	0.0018	0.0014	0.0016b
Deep ploughing	0.0021	0.0012	0.0017b
Mean	0.0018a	0.0015a	

TABLE II. EFFECT OF LEVEL OF  $^{137}\text{Cs}$  AND COUNTERMEASURE ON THE SOIL-TO-CROP TF ( $\text{m}^2 \text{kg}^{-1}$ ) IN ONION SHEATHS

Countermeasure	Levels of $^{137}\text{Cs}$		Mean
	3.7 ( $\text{MBq m}^{-2}$ )	7.4 ( $\text{MBq m}^{-2}$ )	
None/control	0.0032	0.0017	0.0024a
FYM	0.0020	0.0010	0.0016b
K fertilization	0.0016	0.0008	0.0012b
Deep ploughing	0.0021	0.0012	0.0016b
Mean	0.0022a <sup>a</sup>	0.0012b	

<sup>a</sup>Means within a column or row followed by different letters are significantly different at  $P = 0.05$ .

## REFERENCES

- [1] STAUNTON, S., LEVACIC, P., Caesium adsorption on the clay-sized fractions of various soils: Effect of organic matter destruction and charge compensating cation, J. Environ. Radioactivity **45** (1999) 161–172.
- [2] LEE, M.H., LEE, C.W., Association of fallout derived  $^{137}\text{Cs}$ ,  $^{90}\text{Sr}$  and  $^{239,240}\text{Pu}$  with natural organic substances in soils, J. Environ. Radioactivity **47** (2000) 253–262.

## NITROGEN CONTRIBUTION OF GREEN MANURE TO CORN ON AN ULTISOL USING $^{15}\text{N}$ METHODOLOGY

NURHAJATI HAKIM

University of Andalas,  
Padang, Indonesia

M. HELAL

Federal Agriculture Research Centre (FAL),  
Braunschweig, Germany

Globally, much more fertilizer N than P or K is applied to crops. Hydrogen gas is a non-renewable component of N fertilizers, which constitutes a problem in increasing their production. Utilization of plant species that fix atmospheric  $\text{N}_2$ , such as legumes, as green manure, is a possible alternative source of N. However, little research has been reported on amounts of N contributed by green manures for corn production on Ultisols.

The objective of this research was to measure N derived from green-manure prunings by corn on Ultisols, and to choose the best species of legume shrub or tree as a source of organic N.

Four species of legume, *Acacia mangium* and *Paraserianthes falcataria* (trees), and *Cassia mimosoides* and *Crotalaria anagiroides* (shrubs) were planted and labelled with  $^{15}\text{N}$  as sources of N for corn cultivation. Green manure (GM) prunings were applied to corn (*Zea mays*) grown for a 60-day period. Determinations of  $\%^{15}\text{N}$  a.e. in the green manure and corn were made by emission spectrometry.

Soil analyses have shown that Ultisols at the Experiment Station, Padang, Indonesia, are very acid, very high in Al-saturation, and poor in organic matter and plant nutrients, particularly N and P. After liming and applying green manure, pH was increased to 4.80, Al saturation decrease to 20%, and soil organic matter and total N increased. Such a soil was chosen for this experiment, since corn is able to grow well on Ultisols, when Al-saturation is less than 40% [1].

Values for N content, total N, and  $\%^{15}\text{N}$  a.e. of the GM prunings are shown in Table I. The prunings were applied at  $10 \text{ t ha}^{-1}$  fresh weight, equivalent to  $3.3 \text{ t}$  dry weight. In previous work we found that *Cassia mimosoides* was able to obtain 91% of its N needs by fixing atmosphere  $\text{N}_2$ , whereas *Acacia mangium* obtained up to 80% from fixation.

Table II shows that dry matter of corn after 60 days of growth, total N content, and N yields were not significantly influenced by the different species of GM. Total N uptake by corn on the Ultisol was  $65$  to  $91 \text{ kg N ha}^{-1}$ , with 17 to 80% derived from green manure (Table III).

The results showed that *Cassia mimosoides* was most promising as a source of green manure as an alternative to mineral N fertilizer; approximately 80% of N uptake by the corn ( $73 \text{ kg N ha}^{-1}$ ) was derived from the prunings.

TABLE I. NITROGEN CONTENT, TOTAL N, AND %<sup>15</sup>N a.e. OF GREEN MANURE CROPS

Legume	%N	Total (kg N ha <sup>-1</sup> )	% <sup>15</sup> N a.e.
<i>Acacia mangium</i>	5.0	165	0.756
<i>Paraserianthes falcataria</i>	5.4	180	0.517
<i>Cassia mimucuides</i>	4.8	157	0.332
<i>Crotalaria anagyroides</i>	4.9	163	0.364

TABLE II. GREEN MANURE EFFECTS ON DRY WEIGHT AND N YIELD OF CORN

Legume	Dry wt. (t ha <sup>-1</sup> )	%N	Total N (kg ha <sup>-1</sup> )
<i>Acacia mangium</i>	3.91a <sup>a</sup>	1.9a	75.1a
<i>Paraserianthes falcataria</i>	4.37a	2.0a	89.1a
<i>Cassia mimucuides</i>	4.46a	2.0a	91.0a
<i>Crotalaria anagyroides</i>	3.38a	1.9a	64.9a

<sup>a</sup>Numbers in the same column followed the same letters are not significant different (HSD  $P=0.05$ ).

TABLE III. NITROGEN CONTRIBUTIONS FROM LEGUME GREEN MANURE TO CORN

Legume	Green manure N absorbed by corn			Efficiency of use of GM N (%)
	<sup>15</sup> N a.e (%)	Ndfgm <sup>a</sup> (%)	Ndfgm (kg N ha <sup>-1</sup> )	
<i>Acacia mangium</i>	0.129a <sup>b</sup>	17a	12.8a	7.8a
<i>Paraserianthes falcataria</i>	0.139a	27a	23.9a	13a
<i>Cassia mimucuides</i>	0.265b	80b	72.6b	46b
<i>Crotalaria anagyroides</i>	0.161a	44a	28.7a	18a

<sup>a</sup>N derived from green manure. <sup>b</sup>Numbers in a column followed by the same letters are not significantly different (HSD,  $P=0.05$ )

### ACKNOWLEDGEMENT

We thank Dr. I. Schmidt, Bundesministerium für Bildung und Forschung (BMBF), for <sup>15</sup>N and emission spectrometry.

### REFERENCE

- [1] HAKIM, N., et al., "Effect of lime, fertilizer and crop residues on production and nutrient uptake of upland rice, soybean and maize intercropping system", Nutrient Management for Food Crop Production in Tropical Farming Systems (van der HEIDE, J., et al., Eds.), IB, Haren (1989).

## THE USE OF $^{32}\text{P}$ AND $^{15}\text{N}$ TO ESTIMATE FERTILIZER EFFICIENCY IN OIL PALM

ELSJE L. SISWORO, WIDJANG H. SISWORO, HAVID RASJID, SYAMSUL RIZAL  
National Nuclear Energy Agency,  
Jakarta

Z. POELOENGAN, KUSNU MARTOYO  
Centre for Oil Palm Research,  
Medan

Indonesia

Improving efficiency of use of fertilizers has attracted a great deal of interest on oil-palm estates because of increasing input costs. It is assumed that higher efficiency of use of fertilizers for estate crops, including oil palm, would result in significant savings and less environmental pollution. One way to enhance efficiency of use of fertilizers by oil palm is to apply them where the most active roots are located.

Previous work has indicated the possibility of determining the most active roots of tea and chinchona by using  $^{32}\text{P}$  [1–4]. In this experiment,  $^{32}\text{P}$  was again used, to determine the locations of the most active roots of oil palm trees. The trees were 8 years old with an average height of 2.5 m and trunk diameter of 25 cm, and with twenty-five leaves. Phosphorus-32 ( $\text{KH}_2\text{PO}_4$ ) carrier-free solution was injected into twenty holes around each tree; a total of 100 mL, total activity of 24.5 mCi  $^{32}\text{P}$ , were injected as shown below:

Distance from trunk (m)	Soil depth (cm)
1.5	5 and 15
2.5	5 and 15

Three replicates were used. Leaves of oil palm trees were harvested at 2 and 3 weeks after  $^{32}\text{P}$  application. The data reported here are from the second harvest. To determine the locations of the most active roots, two leaves were taken at each harvest, namely the seventeenth and nineteenth leaves. Dpm values were determined in 2-g samples.

The locations of  $^{32}\text{P}$  injection around each trees and the parts of each leaf analyzed for dpm are shown in Fig. 1. Results obtained, expressed in dpm, are shown in Fig. 2.

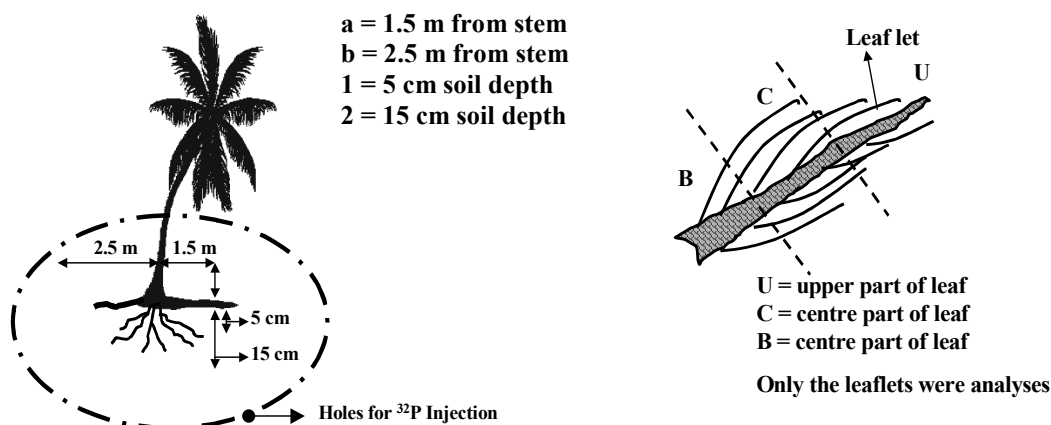


FIG. 1. Locations of  $^{32}\text{P}$  injection and leaf parts analysed for dpm.

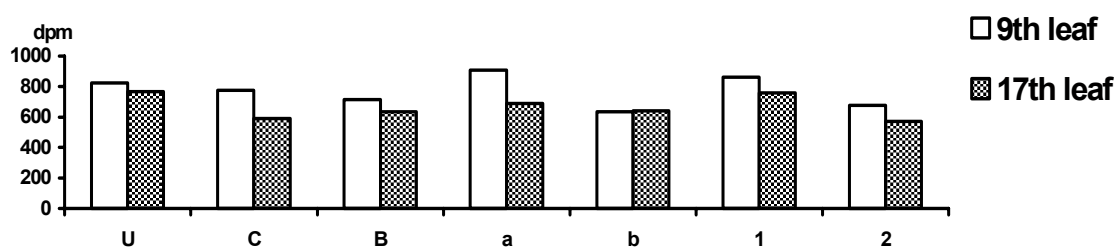


FIG. 2. Dpm values for samples of oil-palm leaves.

Data obtained show that the dpm values for the ninth leaf was generally higher than for the seventeenth leaf. The dpm values from the upper parts of the leaves were highest. The most active roots apparently were located at a distance of 1.5 m from the tree and at a depth of 5 cm.

Accordingly, the efficiency of urea-N use was tested when applied at 1.5 m from each tree trunk and at 5-cm depth. The treatments applied to test the efficiency of urea-N use were as follows:

	1 <sup>st</sup> application		2 <sup>nd</sup> application		3 <sup>rd</sup> application	
	Urea	<sup>15</sup> N-AS	Urea	<sup>15</sup> N-AS	Urea	<sup>15</sup> N-AS
	(g)					
No	0	20	0	20	0	40
N1	1,000	20	0	20	0	40
N2	500	20	500	20	0	40
N3	400	20	300	20	300	40

The second and third applications were made at 4 weeks after each previous application; each tree received 1,000 g of urea. The ninth and seventeenth leaves were harvested, as in the <sup>32</sup>P experiment. Two harvests were made, at 4 and 8 weeks after the last application. Nitrogen-15 labelled ammonium sulphate (AS), 10.12% <sup>15</sup>N, was used. Nitrogen-15 enrichment determinations were made on 1-g samples of the total leaflets, using a NOI-6 PC <sup>15</sup>N analyser. The N-partitioning calculation was as described by Zapata [5]. Results are presented in Fig. 3.

The highest efficiency of use of urea N was obtained when it was applied as two splits (Fig. 3). The amounts of N derived from <sup>15</sup>N-AS were small, but detectable and useful as a tracer. This work shows clearly that <sup>32</sup>P and <sup>15</sup>N could be detected in small samples, and thus used to determine the location of the most active roots and to determine efficiency of use of N from urea.

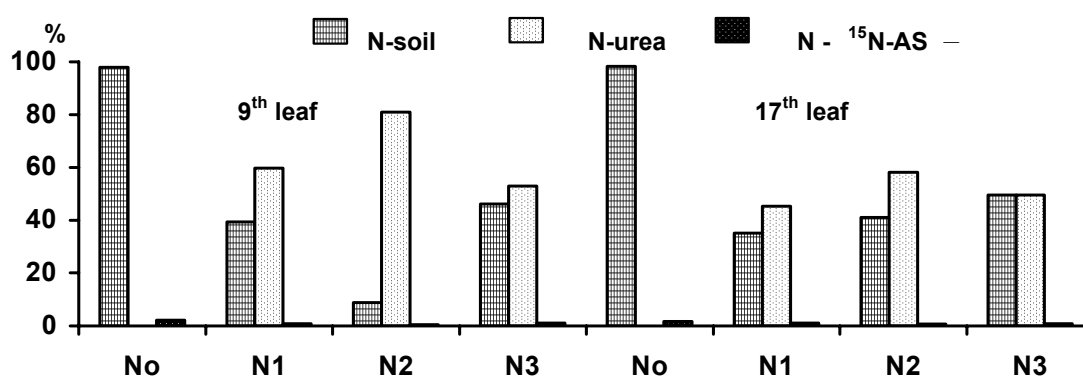


FIG. 3. Partitioning of nitrogen in samples of oil-palm leaves.



## REFERENCES

- [1] SISWORO, E.L., et al., "Studying root distribution of tea using nuclear techniques", Seminar on Applications of Nuclear Techniques, Jakarta, 9-10 July 1985, Centre for Oil-Palm (RISPA), Medan (1986) 279–290. (in Indonesian)
- [2] SISWORO, E.L., et al., Choosing plant parts to be used in root pattern determination of *Chinchona ledgeriana*, Moens, Indon. J. Trop. Agric. **1** (1989) 17–19.
- [3] DARMAWIJAYA, M.I., et al., "Root patterns activity of productive tea shrubs KLON TRI 2025 on latosol soil," Seminar on Applications of Nuclear Techniques, Jakarta 30–31 October 1990, BATAN (Batan Tenaga Atom Nasional/National Atomic Energy Agency), Jakarta (1991) 237–245. (in Indonesian)
- [4] SANTOSO, J., et al., "Patterns of active roots of chinchona after stumping", Seminar on the Applications of Isotopes and Radiation, Jakarta, 30–31 October 1990, Agriculture Institute Bogor (IPR), Bogor. (1991) 157–178. (in Indonesian)
- [5] ZAPATA, F., "Isotope techniques in soil fertility and plant nutrition", Use of Nuclear Techniques in Studies of Soil-Plant Relationships, IAEA-TCS-2, Vienna (1990) 61–128.

## THE USE OF NUCLEAR TECHNIQUES FOR OPTIMIZING FERTILIZER APPLICATION TO IRRIGATED WHEAT

L.K. HENG

FAO/IAEA Agriculture and Biotechnology Laboratory,  
Seibersdorf, Austria

P. MOUTONNET

Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture,  
International Atomic Energy Agency, Vienna

Although wheat is a major cereal, yields are poor in many countries when grown under rainfed conditions. Irrigation helps to increase yields, however, zeal for higher productivity has led to inefficient use of water, nutrients, and pesticides. Recognizing the importance and seriousness of the problem in developing countries, the Joint FAO/IAEA Division implemented a Co-ordinated Research Project (CRP) on “The use of nuclear techniques for optimizing fertilizer application under irrigated wheat to increase the efficient use of nitrogen fertilizer and consequently reduce environmental pollution,” to investigate fertilizer-N uptake efficiency of wheat crops under irrigation, using  $^{15}\text{N}$  and the soil-moisture neutron probe, to determine the fate of applied N, to increase nitrogen- and water-use efficiencies in wheat-cropping systems, and to reduce environmental pollution.

A database was developed and CERES-Wheat, within the decision support system of DSSAT (Decision Support System of Agrotechnology Transfer), was used to formulate specific management strategies and fertilizer-N-rate recommendation for various production conditions. The project was carried out between 1994 and 1998 through the technical co-ordination of the Soil and Water Management & Crop Nutrition Section of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. Fourteen Member States of the IAEA and FAO participated. This paper presents some of the experimental results.

The participating countries were: Bangladesh, Brazil, Chile, China, Egypt, India, Mexico, Morocco, Romania, Syria, and Turkey. The experiments were set up at research stations except for a few carried out on farmers' fields. In general, the fertilizer-N applications represented 0%, 50%, and 150% of the regional recommended rates. The timing of fertilizer application was also studied.

The texture of the soils of the participating countries varied from very sandy in Bangladesh, China, Egypt (in the newly reclaimed soil), India, Mexico and Morocco, to clayey in Brazil, Egypt (in the old irrigated clay soil of the Nile Valley), and Turkey. This difference strongly influenced water-holding capacity and consequently the potential for leaching of nutrients. The organic matter content ranged from very high (7.9%) in Chile for a volcanic ash soil to very low in India and Syria (less than 1%).

Vast differences in rainfall distribution existed between seasons and countries over the 4-year period. Bangladesh, China, and India received large quantities of rainfall, e.g. 1,000 to 2,500 mm recorded in Bangladesh. However, much of this rain occurs outside the wheat-growing season (Bangladesh and India), consequently irrigation is needed. In the case of Morocco, although there is plenty of rain in the growing season, it occurs mostly during the early development of the crop when the requirement for water is small. On the other hand, near zero annual rainfall was recorded in Egypt. In general, most of the countries received rainfall less than 500 mm and, consequently, supplemental irrigation was needed for adequate crop growth. Further details are given in Ref. [1].

Various amounts of irrigation were applied in each country. Brazil, China and Egypt applied the most irrigation (over 450 mm/year) whereas only 75 mm was applied in Bangladesh. Some countries, such as India and Mexico, applied pre-season irrigation; between 200 and 220 mm was being applied in Mexico.

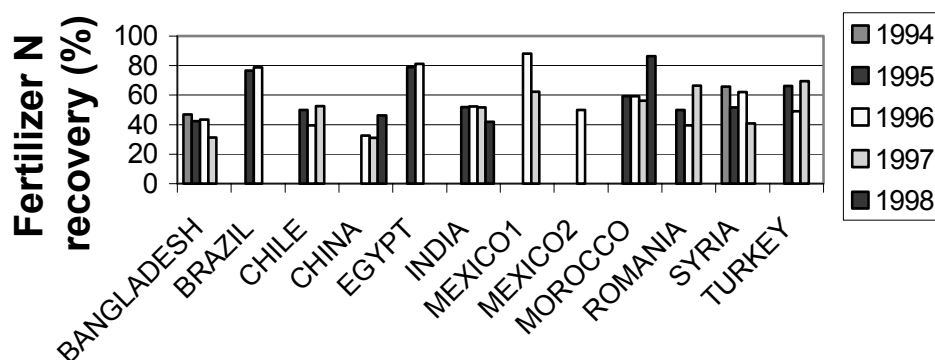


FIG. 1. Fertilizer-N recovery values.

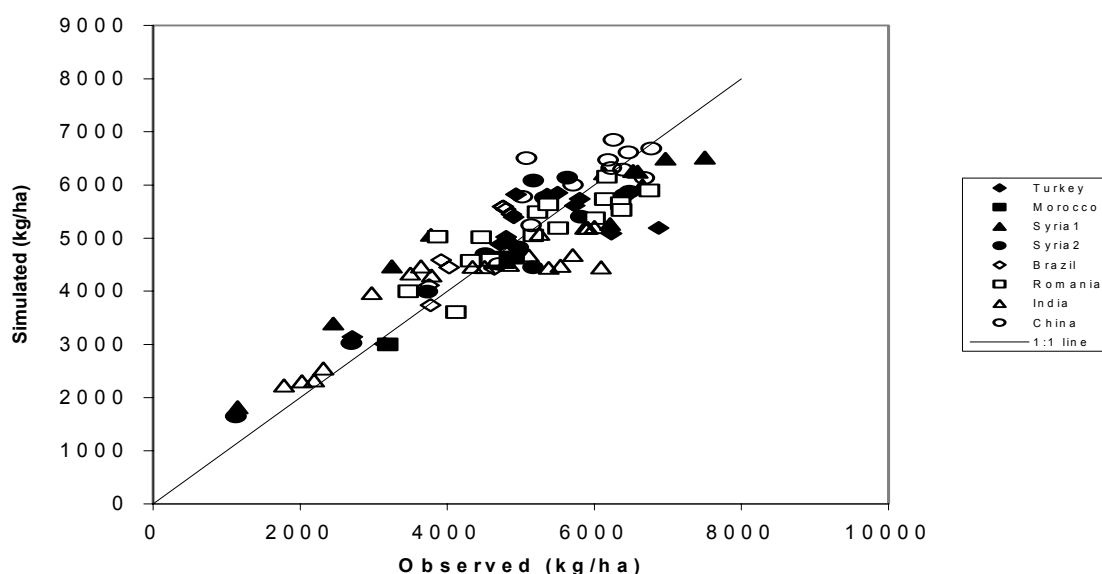


FIG. 2. Observed grain yields and those simulated using CERES-Wheat.

Seven out of the twelve countries use surface irrigation: Bangladesh, China, Egypt, India, Mexico1, Mexico2, and Morocco. The irrigation method, amount and frequency of application, together with the type of soil, determine the efficiency of the applied water.

The amounts of fertilizer recovered in grain and straw from the  $^{15}\text{N}$  applied are shown in Fig. 1. The fertilizer-N recovery values for most countries were high (more than 60%). Those with low fertilizer-N recoveries were: Bangladesh, Chile, China, and sometimes in Romania and Syria, depending on the season. Low recoveries were due to losses mainly through leaching on sandy soils. Interestingly, Chile and China achieved grain yields of 6 to 8 t ha<sup>-1</sup>. In general, the two-thirds split application (applied at the end of tillering) was recovered more efficiently than was the first one-third split application of N fertilizer (applied at planting).

The dataset was used to test the CERES-Wheat model. Figure 2 shows good agreements between observed and predicted grain yields. Good agreements were observed also for other parameters. The ability of the model to predict these parameters implies that it is possible to use this as a tool in facilitating the screening of cultivars for selecting those that are best adapted to specific target environments. This can help in optimizing the use of resources and quantifying risks associated with variations in plant, soil, and weather.

In conclusion, the first one-third split application of fertilizer N (at planting) was usually recovered less efficiently than the second two-thirds split application (at the end of tillering). This observation was consistently made in seven countries during the 1995–98 period (Bangladesh, Brazil, India, Morocco, Romania, Syria, Turkey); therefore, the relative amount applied in the second split may be increased.

Losses of irrigation water and N-fertilizer were observed in Egypt and China on sandy soils and in India; on the other hand, well scheduled sprinkler irrigation promoted fertilizer-N recovery in Syria.

The Ceres-Wheat growth-simulation model closely predicted the progress of dry-matter production, leaf area index, seasonal evapotranspiration, phenological development, and many other plant-growth attributes (Turkey, Syria, Morocco, Brazil, Romania, India).

#### **REFERENCE**

- [1] INTERNATIONAL ATOMIC ENERGY AGENCY, Optimizing nitrogen fertilizer Application to Irrigated Wheat, IAEA-TECDOC-1164, Vienna (2000).

## ASPECTS OF FERTILIZER-N RECOVERY AND BALANCE IN FERTIGATED POTATO IN CENTRAL BEKAA, LEBANON

T. DARWISH

National Council for Scientific Research, Beirut

T. ATALLAH

Lebanese University, Beirut

S. HAJHASAN, A. CHRANEK

Agronomic Research Institute,  
Tel-Amara, Bekaa

Lebanon

Potato, a major spring and summer crop in the Bekaa valley, is known to have high requirements for N and K. Customary farm practice consists of soil application of 450 to 500 kg N/ha for average yields of 20 to 25 t/ha of fresh tubers. Consequently, water quality is threatened as shown by annual increases in nitrate concentration in the groundwater. Thus, for sustainable management one of the tools is the implementation of a combined application of water and fertilizers (fertigation) by drip irrigation. In this work, N requirements by fertigation were studied by comparing four levels of applied N. In 1997, these were, zero, 240, 360 and 480 kg N/ha, and, in 1998, zero, 120, 240 and 360 kg N/ha. The control consisted of soil application at 360 kg N/ha in 1997 and 240 kg N/ha in 1998. Fertilizer-N use efficiency was determined by the isotope-dilution technique in microplots receiving ammonium sulphate enriched in  $^{15}\text{N}$  (1.5% a.e.). Nitrogen balance was evaluated in terms of fertilizer-N recovery by the crop, by the difference and isotope-dilution methods.

Fertilizer N use efficiency varied between seasons, with higher values in 1998 (Table I). The fertigated treatment was comparable to the soil application in 1997, which agrees with a result from the Jordan valley [1]. This was explained by a high volatilization or significant retention of ammonium by the clay particles. For both seasons, no significant differences were obtained in commercial yield. The absence of N did not lead to significant losses in tuber production (data not shown) due to the irrigation water, which contributed 38 kg N/ha (Table I). The remaining N originated from the soil: mineral N and the mineralization of soil organic matter. Soil nitrate-N increased in the 0- to 20-cm layer between the beginning and the end of the experiment, but decreased slightly in the 20- to 60-cm layer. The overall build-up in the soil (bulk density: 1.29) was +19 kg N/ha in 1997 and +41 kg N/ha in 1998.

Mineralized organic N contributed significantly to the balance in the zero-N treatment. Assuming a seasonal rate of mineralization of 1.5%, mineral N (N: 0.147%) reached 114 kg N/ha in the 0- to 40-cm layer. These values match closely the calculated soil-N contribution (Table I). Nitrogen fertilizer contributions based on the difference and isotope-dilution methods were not comparable (Table II), nor were the rankings of treatments, which confirmed some observations [2] and disagreed with others [1]. The higher apparent recovery of fertilizer N in the isotope-dilution method resulted from the large N pool in water and soil. With soil-application, the results were not consistent between the two seasons, which suggests the role of climatic parameters in fertilizer availability and its interception by crop roots. In fact, root distribution in the soil could be another source of difference between the zero-N and the fertilized treatments. Although potato is known to have a shallow rooting system in the absence of N, the volume occupied by roots increased, as indicated by readings with the neutron probe. Also, the soil-N contribution increased (Table I). The soil-applied fertilizer presented similar N yields with the corresponding fertigated treatment, but the fate of N fertilizer was quite different. Thus, the fertigation technique allows a greater availability of the fertilizer and a more efficient removal by the. Soil-N build-up, as a sum of mineralized N and fertilizer N, was more important in 1997 and ranged from 200 kg N/ha (in N1) to 460 kg N/ha (in N3). A moderate level such as N1 in 1998 is recommended as the N build-up was smallest.

TABLE I. NITROGEN YIELD IN POTATO AT PHYSIOLOGICAL MATURITY AND ITS ORIGIN (kg N/ha) BASED ON THE ISOTOPE-DILUTION TECHNIQUE

Season	Treatment	Origin of N in crop (kg N/ha)			N yield (kg N/ha)	Fertilizer input–uptake (kg N/ha)
		Fertilizer	Irrigation water	Soil N		
1997	Zero	—	38	118	156	—
	240 kg N/ha	64	38	88	190	176
	360 kg N/ha	110	38	76	224	250
	480 kg N/ha	103	38	28	169	377
	Con.–soil–360	134	38	64	236	226
1998	Zero	—	55	90	145	—
	120 kg N/ha	108	55	34	197	12
	240 kg N/ha	133	55	58	246	107
	360 kg N/ha	150	55	46	251	210
	Con.–soil–240	63	55	111	229	177

TABLE II. CONTRIBUTION OF FERTILIZERS ACCORDING TO THE DIFFERENCE AND ISOTOPE DILUTION METHODS TO N YIELD IN POTATO

Season	Treatment	N yield (kg N/ha)	Fertilizer-N contribution (kg N/ha)	
			Difference	Isotope dilution
1997	Zero N	156	—	—
	240 kg N/ha	190	34	64
	360 kg N/ha	224	68	110
	480 kg N/ha	169	13	103
	Control–soil–360	236	80	134
1998	Zero N	145	—	—
	120 kg N/ha	197	52	108
	240 kg N/ha	246	101	133
	360 kg N/ha	251	106	150
	Control–soil–240	229	84	63

## ACKNOWLEDGEMENTS

This work was within the regional technical cooperation project RAW/5/002 on “Water Balance and Fertigation for Crop Improvement” with IAEA, Vienna. Financial support was provided by the Lebanese National Council for Scientific Research.

## REFERENCES

- [1] MOHAMMED, M.J., et al., Yield response and nitrogen utilization efficiency by drip-irrigated potato, *Nutrient Cycling Agroecosyst.* **54** (1999) 243–249.
- [2] ZAPATA, F., “Field experiments in isotope-aided studies,” Training Course Series No. 2 on Use of Nuclear Techniques in Studies of Soil-Plant Relationships, IAEA, Vienna (1990) 35–40.

## RECYCLING OF CROP-RESIDUE N FOR SUSTAINABLE PRODUCTION IN A MAIZE-GROUNDNUT ROTATION SYSTEM

ROSENANI ABU BAKAR, SITI ZAUYAH DARUS, MUBARAK ABDELRAHMAN ABDALLA  
Universiti Putra Malaysia (UPM),  
Serdang, Malaysia

To investigate the contribution of crop residues to the N-economy of a maize-groundnut rotation, a long-term field experiment was established in February 1997. It consisted of the following treatments: (i)  $T_1$  – the recommended rate of chemical fertilizer plus residues, (ii)  $T_2$  – the recommended rate of chemical fertilizer without residues, and (iii)  $T_3$  – a combination of organic fertilizer (chicken dung), chemical fertilizer, and residues. In order to investigate the possible contribution of residue N to subsequent crops, the first maize crop was labelled with  $^{15}\text{N}$ . The first crop was sown in March 1997 and  $^{15}\text{N}$ -labelled ammonium sulphate (9.9%  $^{15}\text{N}$  a.e.) was applied at  $60 \text{ kg N ha}^{-1}$  to the  $T_1$  and  $T_2$  treatments, in microplots ( $4 \times 4 \text{ m}$ ) within yield plots ( $20 \times 8 \text{ m}$ ), to generate labelled maize residues. At the same time,  $90 \text{ kg N ha}^{-1}$  unlabelled N fertilizer was applied to provide the locally recommended rate for maize of  $150 \text{ kg N ha}^{-1}$ . Treatment  $T_3$  was included for comparison of yields and appraisal of effects on soil properties. After the first crop was harvested, the labelled aboveground residues in  $T_1$  were applied to a different microplot so that the fate of the labelled residue-N could be followed. Groundnut was grown in rotation with maize, and, after each harvest, the labelled aboveground residues in the microplots of  $T_1$  and  $T_2$  were removed and unlabelled residues were added to  $T_1$ .

Because variability between replicates was high in the treatment  $T_2$ , the average yields in plots with residues,  $T_1$  and  $T_3$ , in the second and third crop cycles were not significantly different from those without residues (Fig. 1). Maize yields were similar in the first and second years.

However, there was a decrease in maize yield in the  $T_2$  plots in the second year. No significant difference between treatments in yield of groundnut (second crop) was observed, presumably because of adequate N supply through  $\text{N}_2$ -fixation. The fourth crop was badly damaged by wild boar. The fifth crop also did not show a significant difference in yield between the  $T_1$  and  $T_2$  plots, but  $T_3$  plots, with combination of chemical fertilizer, chicken dung and residues, gave a significantly ( $P < 0.05$ ) higher yield than  $T_1$  and  $T_2$ .

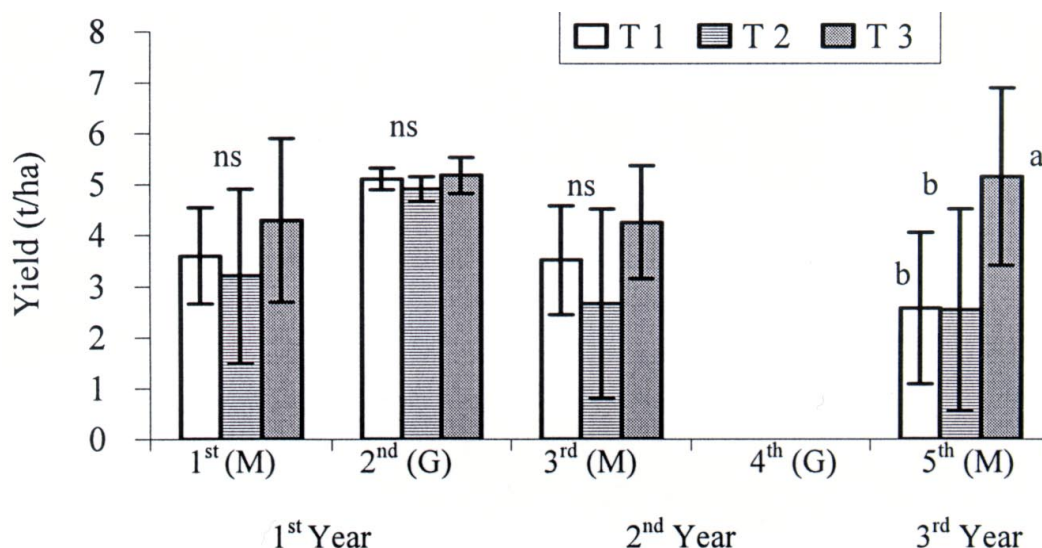


FIG. 1. Yields of four crop cycles (the fourth crop failed due to damage by wild boars). Yields of means with the same small letters do not differ ( $P \leq 0.05$ ); ns = no significant difference between means within crop-year.

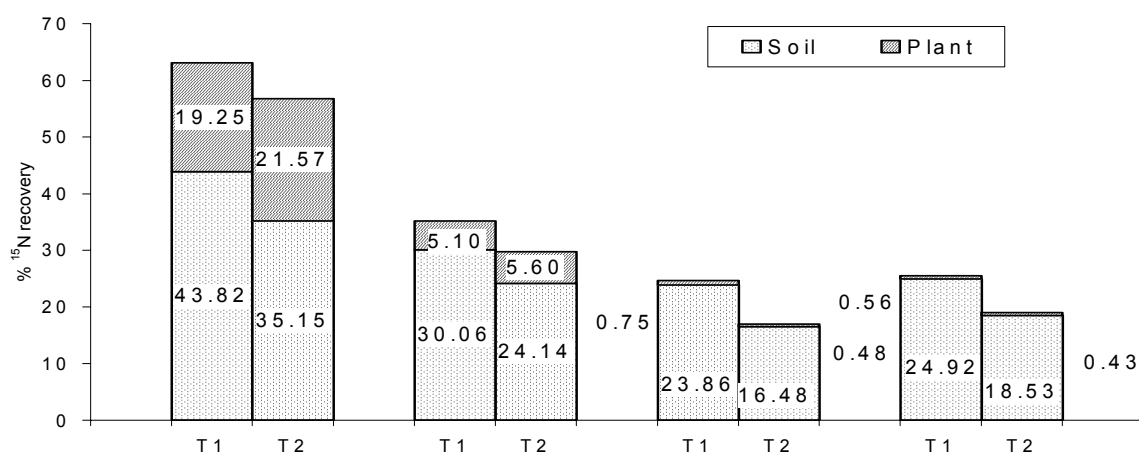


FIG. 2. Recovery of <sup>15</sup>N-labelled fertilizer.

Uptake of <sup>15</sup>N by the first crop of maize was only 19%, while 44% remained in the top 50 cm of the T<sub>1</sub> plots (Fig. 2). In the second crop cycle, the <sup>15</sup>N uptake by the crops was 5.1% while 30% remained in the soil, a recovery totalling 35% compared to 30% in the T<sub>2</sub> plots. In general, most of the <sup>15</sup>N recovered in the soil seemed to be in stable forms as indicated by the substantial amounts remaining after the fourth crop cycle, i.e., 26% and 19% in T<sub>1</sub> and T<sub>2</sub> plots, respectively. In the fifth crop (maize), the %<sup>15</sup>N a.e. values in the crop and soil were too low to give reliable results. The soil analyses at the end of the second year did not show significant effects of the treatments on the total N or organic C contents. The CEC of the soil was slightly higher with T<sub>3</sub> (7.63 cmol kg<sup>-1</sup>) than with T<sub>1</sub> and T<sub>2</sub> (6.19 and 6.20 cmol kg<sup>-1</sup>, respectively), presumably due to the application of the manure. Although <sup>15</sup>N remained in the soil, insignificant amounts were taken up by the crops. This indicates lack of synchrony between N released during decomposition of residues and crop uptake due to long fallow periods (2–3 months). Decomposition of maize residues was found to be rather rapid in the humid tropical conditions, with 50% dry matter lost in 7 to 8 weeks and 40 to 50% of residue-N released in 2 weeks [1].

#### ACKNOWLEDGEMENT

We extend our thanks and gratitude to the International Atomic Energy Agency and the Malaysian Government for providing research funds.

#### REFERENCE

- [1] MUBARAK, A.R., et al., Decomposition and nutrient release from maize (*Zea mays* L.) residues and N uptake by groundnut (*Arachis hypogaea*) in a crop rotation system, Malaysian J. Soil Sci. **93** (1999) 93–107.



## APPLICATION OF SEWAGE SLUDGE FOR CORN CULTIVATION ON A TROPICAL ACID SOIL

ROSENANI ABU BAKAR, CHE FAUZIAH ISHAK  
Universiti Putra Malaysia (UPM),  
Selangor, Malaysia

Malaysia produces about  $5 \times 10^6$  m<sup>3</sup> of domestic sewage sludge per year (wet basis), and this is expected to increase to  $7 \times 10^6$  m<sup>3</sup> by the year 2020 [1]. Thus, there is pressure to develop beneficial means of utilizing this waste. Although domestic in origin, it can have high concentrations of Cu and Zn [2]. A field experiment was established to investigate the utility of applying sludge to agricultural land. The soil at the experimental site is well drained and classified as clayey kaolinitic, isohyperthermic Typic Paleudult, pH 4.7, 0.06% N, and 0.97% organic C. The treatments were: control, inorganic N fertilizer (ammonium sulphate) at 140 kg N/ha, and irradiated (IR) and non-irradiated (NIR) sludge applied at rates equivalent to 0, 150, 300, 450 and 600% of the recommended fertilizer N rate of 140 kg N/ha to the first corn cycle. No sludge was applied during the second corn cycle in order to observe any residual effects of the previously applied sludge. In the third, fourth and fifth corn cycles, the sludge rates were reduced to 100, 200, 300, and 400% of the recommended fertilizer-N rate. The plot size was 4×6 m, and the treatments had four replications. To quantify the availability of sludge N, the indirect <sup>15</sup>N-dilution technique was used. Twenty kg <sup>15</sup>N/ha (10% of a.e.) were added to the control and sludge-treatment plots in the first crop cycle. However, the method failed, as sludge-derived N in the crop could not be calculated. This may have been because the added <sup>15</sup>N was not in equilibrium with soil N when the sludge was added, and the amount was large enough to cause an interaction with the sludge. With the fifth crop, the <sup>15</sup>N technique was applied again, at 5 kg <sup>15</sup>N/ha (20% a.e.) in the all-sludge treatments and 140 kg <sup>15</sup>N/ha at 3% a.e. in the inorganic N-fertilizer treatment.

No significant differences in dry matter yields were found among the various sludge rates, neither were there differences between the IR and NIR sludges due to high variability. In general, it could be observed that sludge applied at 200% N equivalent of the inorganic fertilizer recommended rate was sufficient to give optimum total dry matter yield and economic yield (Fig. 1) as inorganic fertilizer.

Also, there was no significant difference in the concentration of Cu and Zn in the corn grain between IR and NIR sludge treatments for the five cycles and no accumulative uptake of metals in the grain.

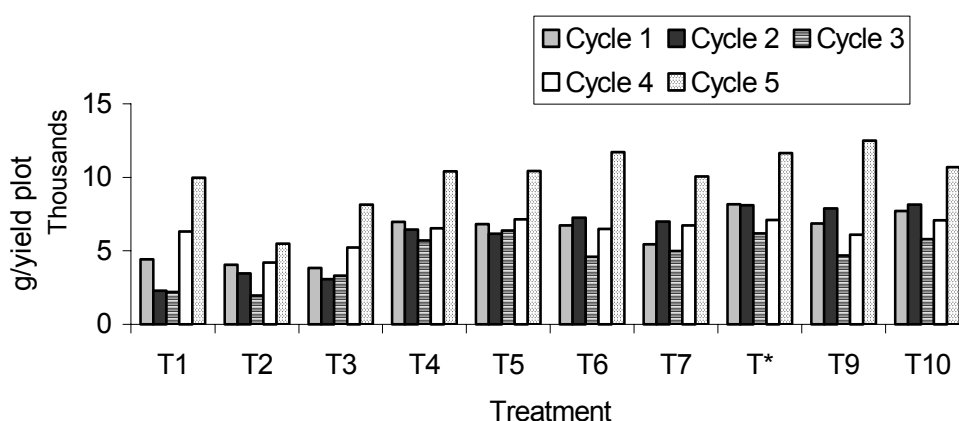


FIG. 1. Mean Economic Yield of 5 crop cycle (T1: Recommended inorganic fertilizer, T2: Control, T3, T4, T5 and T6: IR sludge at 0, 150, 300, and 600% recommended rates respectively in the first crop cycles and 0, 100, 200, 300, and 400% in the third, fourth, and fifth crop cycles, T7, T8, T9 and T10: NIR sludge at the same rates as the IR sludge).

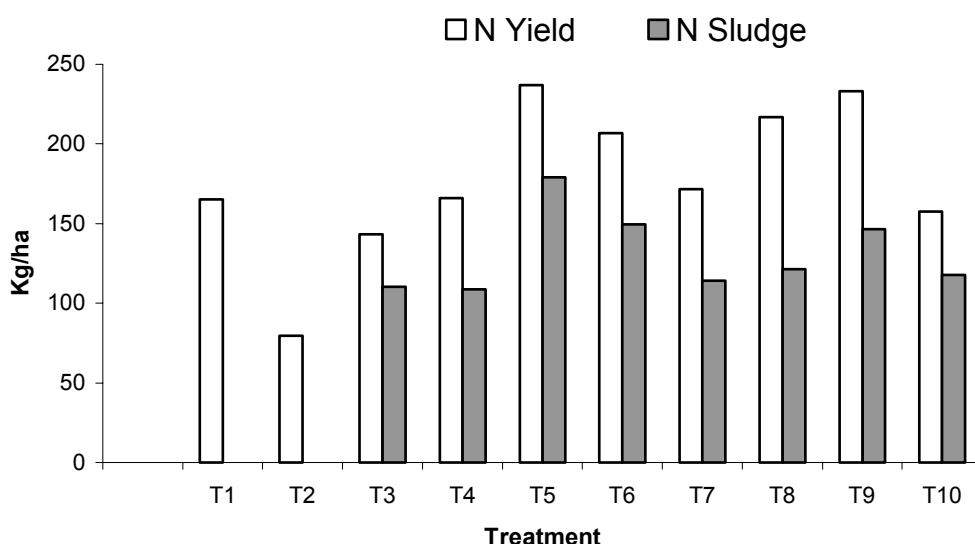


Fig. 2. Total N uptake and N derived from irradiated (T3, T4, T5, T6) and non-irradiated sewage sludge (T7, T8, T9, T10) in the fifth crop of maize.

There was, however, an increase in the accumulation of Cu and Zn in the soil. In the fourth and fifth crop cycles it could be observed that, in general, N uptake increased with sludge-rate application up to the equivalent of 300% of the recommended inorganic fertilizer-N rate, and N uptake decreased considerably with the higher rates. This indicates an inhibitory effect possibly due to net N immobilization with higher organic matter addition. Figure 2 shows the total N and N derived from sludge that had been added up to the fifth crop; the soil supplied about 56 kg N/ha to the crop and 62 to 77% of the applied chemical fertilizer  $^{15}\text{N}$  was assimilated.

#### ACKNOWLEDGEMENTS

We extend our thanks and gratitude to the International Atomic Energy Agency, Vienna, and the Malaysian Government for providing research funds.

#### REFERENCES

- [1] ANONYMOUS, A Pamphlet on a Potty History of Sewage Sludge and Its Treatment, Indah Water Consortium, Kuala Lumpur (1997).
- [2] McGRATH, S.P., et al., Long-term effects of metals in sewage sludge on soils, microorganisms and plants, J. Industrial Microbiol. **14** (1995) 94–104.

## THE POTENTIAL OF FRESH LEAVES TO IMPROVE ACID-SOIL INFERTILITY

ZAHARAH A. RAHMAN  
Universiti Putra Malaysia,  
Serdang

W.A.K. WAN RASHIDAH  
Forest Research Institute,  
Kuala Lumpur

Malaysia

The amounts of nitrogen fixed by six leguminous tree species (*Gliricidia sepium*, *Parkia speciosa*, *Azadirachta excelsa*, *Paraserianthes falcataria*, *Acacia mangium* and *Leucaena leucocephala*) were measured over a 30-month period, using the  $^{15}\text{N}$ -dilution method [1] and two non-fixing checks (*Hopea odorata* and *Khaya ivorensis*). *Paraserianthes falcataria* was found to be the fastest growing and the highest in  $\text{N}_2$ -fixing ability.

The potential of green leaves of *P. falcataria* to function as an ameliorant of acid tropical soils was studied using polyvinyl leaching tubes [2] with an anion-cation resin bag placed at the bottom end. The leaching tubes, 8 cm in diameter and 25 cm in length, were inserted into the soil and immediately taken out with the column of soil inside. A bag containing 25 g of mixed resin was placed at the lower end of each hole, and the tubes were immediately reinserted into the soil. Fresh leaves (25-g aliquots) of *P. falcataria* were placed on the soil surface (as mulch) within the tubes or were incorporated into the top 20 cm of the soil. Four tubes were sampled randomly at 3, 5, 10, 20, 30, 40, 50, 60, and 70 days later. The soil in each tube was divided into the top 10 cm and the bottom 10 to 20 cm and the resin bag was also sampled and analysed for mineral N, exchangeable bases, and exchangeable Al.

Mineral N build-up in the soil was higher in the leaf-incorporated than in the leaf-mulch treatment (Fig. 1). Mulching resulted in a greater build-up of exchangeable Ca and was effective in reducing exchangeable Al saturation (93%) with a concomitant increase in pH in the top soil (data not shown). Leaching of nitrates was more pronounced in the mulched treatment than incorporated, while losses of Ca were more pronounced in the incorporated treatment (data not shown).

## REFERENCES

- [1] DANSO, S.K.A., et al., Biological nitrogen fixation in trees in agro-ecosystems, *Plant Soil* **141** (1992) 177–196.
- [2] SUBLER, S., et al., Comparison of buried bag and PVC core methods for in-situ measurements of nitrogen mineralisation rates in an agricultural soil, *Commun. Soil Sci. Plant Anal.* **26** (1995) 2369–2381.

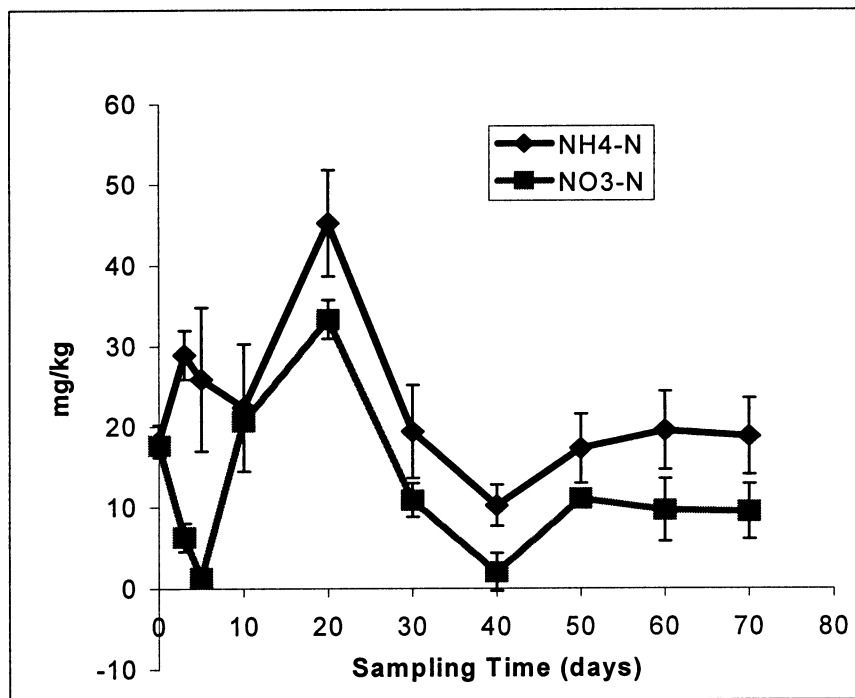
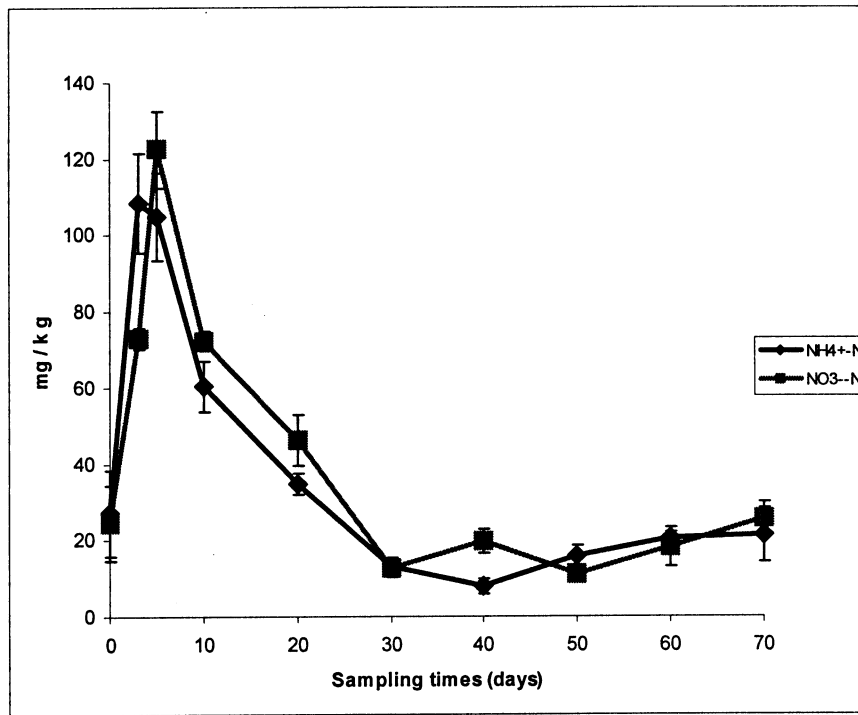


FIG. 1. Temporal trends in  $\text{NH}_4\text{-N}$  and  $\text{NO}_3\text{-N}$  content in the top 0- to 10-cm layer of soil after *P. falcataria* leaves were incorporated (upper) or applied as mulch (lower).

## USE OF $^{15}\text{N}$ AND THE NEUTRON PROBE IN EVALUATING SOIL ORGANIC MATTER TURNOVER AND WATER MANAGEMENT IN WHEAT

M. ISMAILI

Université Moulay Ismail,  
Meknés

A. ICHIR

Université Moulay Ismail,  
Errachidia

Morocco

An experiment was conducted at the experimental station of the Regional Office of Agricultural Development in South Morocco (Errachidia). The soil is a sandy loam, and average rainfall is 50 mm/yr. Temperatures are relatively low in winter ( $-3^{\circ}\text{C}$ ) and high in summer ( $45^{\circ}\text{C}$ ). The soil pH was 8.4, with 0.069% N, 0.97% O.M., 5 ppm exchangeable K, 8.8 ppm available P, and 0.25 exchange capacity.

Wheat (cv. Massa) was grown on land previously amended with N, P, and K (42, 84, and 42 kg/ha, respectively). Three irrigation treatments were imposed: 20% HCC (soil humidity at 20% of field capacity), 40% HCC, and 60% HCC. Water treatments were maintained by measurement of soil moisture with a neutron probe. Within each watering system, two N treatments were used: 835 g/m<sup>2</sup> of wheat residues enriched with 1.711% atom excess  $^{15}\text{N}$  (105 mg  $^{15}\text{N}/\text{m}^2$ ) at seeding, and 4.10 g N/m<sup>2</sup>, as ammonium sulphate, a month after seeding; 4.10 g N/m<sup>2</sup> ammonium sulphate, enriched with 9.96% atom excess  $^{15}\text{N}$  after seeding and another 4.10 g N/m<sup>2</sup> enriched in  $^{15}\text{N}$  (9.96%) a month later (836 mg  $^{15}\text{N}/\text{m}^2$ ).

The hydroprobe was calibrated under dry- and humid-soil conditions (Table I). Soil samples were taken at various depths (50 cm from the access tubes) to determine soil humidity by drying samples at  $105^{\circ}\text{C}$  for 24 h. Apparent density was determined to calculate soil volumic humidity at the same depths by the cylinders method. Then volumic humidity was correlated with the hydroprobe count ratio (direct count/standard count) (Table II).

Yields and  $^{15}\text{N}$  enrichment of seeds, residues, and roots were determined. Soil N and  $^{15}\text{N}$  were determined at four depths to determine the fate of residue  $^{15}\text{N}$  and fertilizer  $^{15}\text{N}$  added to the soil. The results are summarized in Tables III and IV. A simple mathematical model allowed us to calculate all parameters needed to understand the soil-water relationship with N budget. The use of the neutron probe allowed maintenance of watering levels at particular depths.

Water deficit affects wheat yield, and residue production. One irrigation, every 15 days, during the growing season doubled yield and residue production. Higher yields were obtained by keeping soil humidity at 60% HCC (one irrigation per week). Under 20% HCC irrigation, yield and total N were higher with wheat residues applied than with fertilizer N, whereas, with 40% HCC, fertilizer N and residue treatments gave similar results. With 60% HCC, fertilizer N produced more yield than did wheat residues.

The effect of residues on  $^{15}\text{N}$  accumulation (3.2 to 6.9% recovery) by wheat was small compared to that from fertilizer  $^{15}\text{N}$  (19 to 33% recovery), but effects on yield were similar in both treatments. The results of this experiment are a model for a number of experiments to be conducted on long-term effects of organic matter addition to soil, on various crops in different regions of Morocco.

TABLE 1. CALIBRATION CURVES OF THE HYDROPROBE

Depth (cm)	Calibration curves	Correlation Coefficient
0–15	HV1 = $-3.760 + 23.126 R_1$	$R^2=0.98$
15–30	HV2 = $-7.549 + 24.113 R_2$	$R^2=0.93$
30–45	HV3 = $-3.238 + 20.430 R_3$	$R^2=0.99$
45–60	HV4 = $-0.516 + 18.437 R_4$	$R^2=0.95$
60–75	HV5 = $-0.669 + 18.381 R_5$	$R^2=0.98$
75–90	HV6 = $-1.006 + 17.461 R_6$	$R^2=0.97$

TABLE II. VOLUMIC HUMIDITY AND CORRESPONDING COUNT RATIO NECESSARY FOR EACH IRRIGATION TREATMENT

Soil depth	Dry soil		20% HCC		40% HCC		60% HCC		Field capacity	
	V.H.	C.R.	V.H.	C.R.	V.H.	C.R.	V.H.	C.R.	V.H.	C.R.
0–15	8.38	0.50	16.8	0.87	17.9	0.95	19.7	1.09	31.9	1.50
15–30	10.2	0.68	18.5	1.18	20.6	1.23	24.3	1.32	30.5	1.47
30–45	10.7	0.68	19.1	1.12	20.4	1.18	22.1	1.19	24.8	1.37
45–60	10.2	0.56	18.5	1.14	20.5	1.14	21.8	1.18	24.7	1.31
60–5	8.25	0.48	19.5	1.15	21.1	1.18	21.8	1.24	23.1	1.23
75–90	7.15	0.46	17.7	1.13	18.6	1.14	22.2	1.23	21.4	1.31

TABLE III. YIELD, RESIDUE, AND ROOT DRY WEIGHTS, N AND  $^{15}\text{N}$  DATA FOR WHEAT GROWN WITH THREE IRRIGATIONS (TREATMENT 1, RESIDUES APPLIED AT 835 g/m<sup>2</sup>)

Irrig'n (% HCC)	Yield (kg/ha)	Resid. (kg/ha)	Root (kg/ha)	Plant N (kg/ha)	Seed % $^{15}\text{N}$ exc.	Resid. % $^{15}\text{N}$ exc.	Root % $^{15}\text{N}$ exc.	Plant T. $^{15}\text{N}$ (mg/m <sup>2</sup> )	Soil $^{15}\text{N}$ (mg/m <sup>2</sup> )	Non account (mg/m <sup>2</sup> )	$^{15}\text{N}$ recov (%)
20	1,842	2,486	219	84.3	0.042	0.032	0.042	3.36	60.5	41.8	3.2
40	3,925	5,840	333	147	0.049	0.051	0.070	7.34	40.7	57.6	6.9
60	5,440	7,697	550	193	0.025	0.021	0.036	4.60	78.4	22.7	4.4
LSD	207	373	38	15.6				ns			

TABLE IV. YIELD, RESIDUE, AND ROOT DRY WEIGHTS, N AND  $^{15}\text{N}$  DATA FOR WHEAT GROWN WITH THREE IRRIGATIONS (TREATMENT 2. WITH N FERTILIZER (8.4 g N/m<sup>2</sup>))

Irrig'n (% HCC)	Yield (kg/ha)	Resid. (kg/ha)	Root (kg/ha)	Plant N (kg/ha)	Seed % $^{15}\text{N}$ exc.	Resid. % $^{15}\text{N}$ exc.	Root % $^{15}\text{N}$ exc.	Plant T. $^{15}\text{N}$ (mg/m <sup>2</sup> )	Soil $^{15}\text{N}$ (mg/m <sup>2</sup> )	Non account (mg/m <sup>2</sup> )	$^{15}\text{N}$ recov (%)
20	1,492	1,988	246	75.9	2.015	2.17	1.862	1560	139	64.7	18.7
40	4,569	6,092	392	192	1.504	1.228	1.658	272	148	49.8	32.5
60	6,384	8,940	907	214	1.194	1.47	1.388	275	108	54.6	32.9
LSD	260	299	24	20.4				44.2			

## ACKNOWLEDGEMENTS

We gratefully acknowledge the financial support of the IAEA. We thank Dr. Felipe Zapata and Dr. Gamini Keerthisinghe for their advice, and the staff at the Seibersdorf Laboratory for  $^{15}\text{N}$  analysis.

## REFERENCES

- [1] Glendining, M.J., et al., Fate of  $^{15}\text{N}$ -Labelled fertilizer applied to spring barley grown on soils of contrasting nutrient status, *Plant Soil* **195** (1997) 83–98.

## EFFECT OF N AND P AND THEIR INTERACTIVE EFFECTS WITH RICE GENOTYPES ON N<sub>2</sub> FIXATION

R.K. SHRESTHA

Nepal Agriculture Research Council,  
Lalitpur, Nepal

J.K. LADHA

International Rice Research Institute,  
Los Baños, Philippines

A greenhouse pot experiment was conducted at the International Rice Research Institute to examine the effects of exogenous N and P, and their interaction with genotype, on rice-associated biological N<sub>2</sub> fixation. Sieved soil (2 mm) in cement containers (6.5 m length × 2 m width × 0.25 depth) was labelled with (<sup>15</sup>NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> at a rate of 6.3 kg N ha<sup>-1</sup> containing 99.5 atom % excess <sup>15</sup>N.

The soil was then mixed thoroughly with a hand-powered tiller. Subsequent mixing was done three times a week for 30 min over a period of 6 wk to minimize vertical stratification in <sup>15</sup>N enrichment [1]. The soil was kept submerged with 3 to 5 cm of standing waters throughout the 6-wk period. A three-factor experiment (N: 0, 60 and 120 kg ha<sup>-1</sup>; P: 0 and 60 kg ha<sup>-1</sup>; and genotypes: BG380-2, Pankaj, Gogo Putih, OR142-99, Oking Seroni, and Murungakayan 302) was conducted in a randomized complete block design with four replications. Atom % <sup>15</sup>N excess values of plant samples were determined at plant maturity.

The exogenous supply of all levels of fertilizer-N significantly reduced atom % <sup>15</sup>N excess values of the whole plants in all six genotypes. The mean atom % <sup>15</sup>N excess of 0.2212 at 0 kg N ha<sup>-1</sup> was decreased to 0.1593 and 0.1202 with applied N at 60 and 120 kg ha<sup>-1</sup>, respectively (Table I). Similar observations were made by Hardarson et al. [2]. These decreases are attributable to fertilizer-N uptake diluting <sup>15</sup>N enrichment. Atom % <sup>15</sup>N excess values for whole plants were significantly negatively correlated with N uptake ( $r^2=0.948^{**}$ ).

Phosphorus fertilizer did not affect atom % <sup>15</sup>N excess significantly, but slight decreases were generally observed (Table II). Similar observations have been reported in soybean [3].

Significant genotypic differences in atom % <sup>15</sup>N excess were observed when N was not applied (Table I). Oking Seroni had the lowest atom % <sup>15</sup>N excess, consistent with an earlier study [4]. Interestingly, these genotypic differences disappeared with the application of N fertilizer (Table I). In contrast, phosphorus fertilizer application did not suppress genotypic differences <sup>15</sup>N enrichment (Table II).

TABLE I. EFFECT OF LEVEL OF N FERTILIZER ON ATOM %<sup>15</sup>N EXCESS IN RICE (AVERAGES FOR TWO P LEVELS)

Genotype	Atom % <sup>15</sup> N excess		
	0 kg N ha <sup>-1</sup>	60 kg N ha <sup>-1</sup>	120 kg N ha <sup>-1</sup>
BG 382-2	0.2242b <sup>a</sup>	0.1629a	0.1228a
Pankaj	0.2277ab	0.1597a	0.1201a
Gogo Putih	0.2231b	0.1585a	0.1214a
OR 142-99	0.2400a	0.1608a	0.1219a
Oking Seroni	0.2071c	0.1531a	0.117a
Murungakayan	0.2050c	0.1606a	0.1181a
Mean	0.2212A	0.1593B	0.1202C

<sup>a</sup>Numbers followed by the same lower-case or upper-case letter, respectively, are not significantly different ( $P < 0.05$ ) by DMRT.

TABLE II. EFFECT OF LEVEL OF P FERTILIZER ON ATOM %  $^{15}\text{N}$  EXCESS IN RICE (AVERAGES FOR THREE N LEVELS)

Genotype	Atom % $^{15}\text{N}$ excess		Mean
	0 kg P ha <sup>-1</sup>	60 kg P ha <sup>-1</sup>	
BG 382-2	0.1693ab <sup>a</sup>	0.1707ab	0.170ab
Pankaj	0.1763a	0.1620abc	0.1691ab
Gogo Putih	0.1674ab	0.1680abc	0.1677abc
OR 142-99	0.1735ab	0.1750a	0.1742a
Oking Seroni	0.1604b	0.1578bc	0.1591c
Murungakayan	0.1654ab	0.1570c	0.1612bc
Mean	0.1687A	0.1651A	0.1669

<sup>a</sup>Numbers followed by the same lower-case or upper-case letter, respectively, are not significantly different ( $P < 0.05$ ) by DMRT.

Inhibitory effects of exogenous N indicate a limited potential for associative  $\text{N}_2$  fixation to significantly benefit rice productivity. Undoubtedly, non-symbiotic  $\text{N}_2$  fixation in the soil system and symbiotic fixation by legumes in rotation with lowland rice will continue to be crucial in sustaining soil-N status under low- and high-production systems. But, considering the low levels and narrow range of genetic differences in associative  $\text{N}_2$  fixation, particularly in soils with high N and high  $\text{G} \times \text{E}$  interaction, a selection and breeding strategy is not feasible [5].

## REFERENCES

- [1] WATANABE, I., "Errors related to the  $^{15}\text{N}$  dilution method for estimating nitrogen fixation", Stable Isotopes in Plant Nutrition, Soil Fertility and Environmental Studies, IAEA/FAO, Vienna (1991) 83–88.
- [2] HARDARSON, G., et al., Measurements of nitrogen fixation in fababean at different N fertilizer rates using the  $^{15}\text{N}$  isotope dilution and A-value methods, Plant Soil. **131** (1991) 161–168.
- [3] PONGSAKU, P., JENSEN, S., Dinitrogen fixation and soil N uptake by soybean as affected by phosphorus availability, J. Plant Nutr. **14** (1991) 809–823.
- [4] SHRESTHA, R.K., LADHA, J.K., Genotypic variation in promotion of rice dinitrogen fixation as determined by nitrogen-15 dilution, Soil Sci. Soc. Am. J. **60** (1996) 1815–1821.
- [5] MALARVIZHI, P., LADHA, J.K., Influence of available N and rice genotype on associative nitrogen fixation, Soil Sci. Soc. Am. J. **63** (1999) 93–99.



## EFFICIENCY OF USE OF FERTILIZER NITROGEN BY GRAPEVINE CULTIVATED ON SANDS AS INFLUENCED BY TIME OF APPLICATION

G.E. SUTEU

University of Agricultural Sciences,  
Bucharest

A. SERDINESCU

Research Institute for Viticulture and Enology,  
Valea Călugărească

M. TIRCOMNICU

Central Research Station for Agricultural Crops on Sands,  
Dăbuleni

Romania

An accurate recommendation for N fertilization of grapevine (*Vitis vinifera* L.) cultivated on sands is essential for optimum growth and to prevent groundwater pollution. Previous research using  $^{15}\text{N}$  indicated that a single spring application resulted in losses by leaching and serious pollution of groundwater by nitrate, greatly in excess of permitted limits [1]. Two or three split applications of fertilizer N, in autumn and spring, may increase fertilizer-N use efficiency and decrease pollution [2]. The aim of our experiment was to investigate fertilizer N use efficiency and N compartmentation in the annual and perennial organs of grapevine in response to the time of application, by using  $^{15}\text{N}$ .

The experiment was conducted in a 7-year-old vineyard on sands, with cv. Rosioară fertilized with 120 kg N/ha (28 g N/vine) applied in three equal rates of 40 kg N/ha: in the autumn (end of November), in February, and in the first half of May. Each time, only one of the three experimental microplots (five vines) received the N as labelled ammonium nitrate ( $^{15}\text{NH}_4^{15}\text{NO}_3$ ); the other microplots received the same amount non-labelled ammonium nitrate. The  $^{15}\text{N}$  enrichment was 9.90% atom excess for the November application, 5.25% for February, and 4.90% for May. This isotope procedure is the only way of determining the N use efficiency for each of the three times of application.

In autumn, at maturity of the grapes, whole plants from each experimental microplot were sampled for chemical and isotopic analyses (dry matter content, N content, and  $^{15}\text{N}$  excess). Total N determination was made by the Kjeldahl method, and the  $^{15}\text{N}$  excess was determined by mass-spectrometry. Besides the determination of N utilization coefficients, the experimental data allowed determination of the distribution of the total and labelled N in the annual and perennial organs of grapevine.

In order to estimate the quantity of N taken up from soil and from fertilizer by a grapevine plant during the growing season, the N accumulation during the “dormant” period was determined. For this purpose three vines near the experimental microplots were excavated from before bud burst, their perennial organs were collected and analysed for dry matter and total N content. It is noteworthy that these vines had been fertilized in the autumn with 40 kg N/ha as unlabelled ammonium nitrate.

The data show that, of the total amount of N accumulated from bud burst to harvest time, 28.8 g, the largest portion was in the roots, followed by leaves and the trunk (Table I). Nitrogen derived from fertilizer was preferentially accumulated in leaves (Table II), which is attributable to the fact that the leaves play an important role in the synthesis of proteins.

Approximately the same amounts of  $^{15}\text{N}$  were found in roots and trunk at harvest time. Nitrogen is stored chiefly in these organs, especially during the autumn. Doubtless, the amounts would have been greater if the leaves had already senesced. One third to a half of N present in the leaves at the beginning of October is then transferred to roots and trunk for storage until the following growing season.

TABLE I. TOTAL NITROGEN (g) ACCUMULATED IN ORGANS OF GRAPEVINE

Organ	When <sup>15</sup> N applied			Mean
	Autumn	February	May	
Leaves	5.82	5.89	9.27	6.99
Grapes	1.62	1.06	2.24	1.66
1 <sup>st</sup> -year wood	3.02	2.60	4.26	3.29
2 <sup>nd</sup> -year wood	0.74	0.43	0.99	0.72
Perennial wood	0.89	0.86	0.66	0.80
Trunk	7.38	4.94	5.84	6.05
Old roots	5.04	3.56	6.23	4.94
Young roots	4.42	3.20	5.43	4.35
Total/vine	28.9	22.5	34.2	28.8

TABLE II. NITROGEN FROM FERTILIZER (mg <sup>15</sup>N) IN ORGANS OF GRAPEVINE

Organ	When <sup>15</sup> N applied			Total (mg)
	Autumn	February	May	
Leaves	731	955	525	2,211
Grapes	169	158	135	462
1 <sup>st</sup> - year wood	234	412	156	802
2 <sup>nd</sup> -year wood	50	37	18	105
Perennial wood	33	60	10	103
Trunk	242	376	160	778
Old roots	225	308	173	706
Young roots	191	291	228	710
Total/vine	1,875	2,597	1,405	5,877

TABLE III. DRY MATTER AND TOTAL N ACCUMULATED BY ORGANS OF GRAPEVINE BEFORE BUD BURST

Organ	Dry matter (g)	N (%)	N (g)
1 <sup>st</sup> -year wood	95.6	0.51	0.48
2 <sup>nd</sup> -year wood	90.3	0.63	0.57
Perennial wood	168	0.57	0.96
Trunk	566	0.50	2.80
Old roots	251	0.52	1.31
Young roots	336	0.55	1.85
Total/vine	1,507		7.97

The quantity of N accumulated during dormancy was 7.97g (Table III); this means that only 20.8 g of soil and fertilizer N accumulated in a grapevine plant during the growing season; 5.9 g came from the three fertilizer application (see Table II), resulting in an overall efficiency of fertilizer-N use of only 28%. Of this, 32% (1.88 g N) came from the first application (in the autumn), and 44% (2.59 g N), and 24% (1.41 g N), respectively, came from the February and May applications, respectively.

It seems that with late-autumn and early-spring applications of N, the fertilizer-N use efficiency in vineyards cultivated on sands would probably be improved, provided that autumn rains are not sufficient to cause leaching.

#### REFERENCES

- [1] SERDINESCU, A., SUTEU, G.E., N-leaching studies with grapevine in lysimeters, using <sup>15</sup>N, XI-th World Fertilizer Congress (Proc. Symp. Ghent) (1997) 275.
- [2] BANITĂ, P., Viticultura pe nisipuri (CERES Ed.), Bucuresti (1985) 78–82.

## ORGANIC MATTER AFFECTS RETENTION OF APPLIED FERTILIZER NITROGEN

U.R. SANGAKKARA, C.S. KANDAPOLA

University of Peradeniya,  
Peradeniya, Sri Lanka

Soil organic matter is a very important component in tropical cropping systems for maintaining sustainability [1], as demonstrated, for example by studies on rice [2]. However, the impact of organic matter, especially as crop residues, on the availability of added fertilizer is not well defined. Most research has concentrated on the impact of green manures on yields and soil fertility [3]. As nitrogen (N) is the nutrient most abundantly added to tropical crops [1] and is lost easily from the rhizosphere, a long-term field experiment was carried out to determine the effect of organic matter on the availability of  $^{15}\text{N}$ -enriched fertilizer.

The study, carried out within a Co-ordinated Research Project of the International Atomic Energy Agency, Austria, was located at the University of Peradeniya, Sri Lanka. A cereal-based crop rotation consisting of corn (*Zea mays* L.) and mung bean (*Vigna radiata* L. Wilkcec) was used to evaluate the impact of crop residue on the long-term availability of added N to the first crop in terms of yield. The experiment, carried out over four seasons and consisting of two crops each of corn and mung bean, was laid out in a randomized block design with four replicates. The treatments included two plots (8×6 m) with subplots (3×3 m) to which labelled fertilizer was applied, and one without to supply unlabelled residue. At the onset of the first season (April 1997) corn was planted in all plots. The N was applied at a rate equivalent to 60 kg/ha, as  $\text{NH}_4\text{SO}_4$ . The subplots received the same amount of N in the form of 10%  $^{15}\text{N}$ -enriched  $\text{NH}_4\text{SO}_4$ , and the crop was managed per local recommendations. At the end of the first season, seed and residue yields were determined and sub-samples from subplots kept for analysis. Soon after harvest, unlabelled residue from the plot that did not receive  $^{15}\text{N}$  was added to the labelled subplot at a rate equivalent to that removed, and incorporated. Organic matter was not added to the second labelled subplot. Thus, the main treatments developed after the first season were those that received  $^{15}\text{N}$  and were either supplied with unlabelled residue or maintained with crop residues.

In the second season, mung bean was planted and, at maturity, seed and residue yields determined, along with  $^{15}\text{N}$  enrichment. Thereafter, unlabelled residue from the plot that did not receive  $^{15}\text{N}$  was added to the plot that received organic residue at the end of season 1. The other labelled subplot did not receive crop residue. Corn and mung bean were planted in seasons 3 and 4 and the same method adopted. The seed and residue yields were measured in all seasons and the  $^{15}\text{N}$  enrichments determined from sub-samples obtained from the subplots.

The yields of seeds and residue in treatments T1 (organic matter added) and T4 (organic matter removed) were similar, thus illustrating the lack of any differences between treatments at the inception of the study, in season 1 (Table I).

The incorporation or removal of corn residue had no significant impact on the percentage N in mung bean seeds or residue. In contrast, seed and residue yields of mung bean were enhanced by the incorporation of residues. More importantly, the availability of fertilizer N applied in the previous season as denoted by Ndff and % recovery was also significantly improved by the application of residue (T1). This showed the benefits of applying crop residues soon after harvest to retain the applied N and make it available to the next crop. A similar trend was also observed in season 3 in corn with the incorporation of mung bean residue after harvest. Seed and residue yields were greater with the addition of organic matter. Again, the more important factor was the high quantity of enriched N, especially in the seeds, derived from fertilizer applied two seasons prior. This was not very evident in the residue of corn, either in terms of Ndff or % recovery of enriched N. This indicated that the residual fertilizer was stored in the seed rather than in the residue, and this phenomenon warrants further study.

TABLE I. IMPACT OF ORGANIC MATTER ON THE AVAILABILITY OF FERTILIZER N

Season and Crop	Treatment	% N	Yield —— (kg/ha) ——	Ndff	%N Recov.
Season 1					
Corn seed	T1	1.8	4,276	13.9	23
	T4	1.8	4,108	14.1	23
Residue	T1	1.1	14,610	30.2	50
	T4	1.0	14,481	29.5	49
	Sx	0.04	1,422	2.15	3.6
Season 2					
Mung seed	T1	4.47	2,642	1.85	3.09
	T4	4.52	2,104	1.29	2.15
Residue	T1	1.12	5,180	1.17	1.95
	T4	1.18	4,599	0.83	1.38
	Sx	0.03	158	0.11	0.24
Season 3					
Corn seed	T1	1.76	4,015	0.56	0.93
	T4	1.66	2,699	0.33	0.56
Residue	T1	0.78	14,115	0.47	0.78
	T4	0.87	14,028	0.45	0.75
	Sx	0.02	88	0.04	0.33
Season 4					
Mung seed	T1	4.54	3,258	0.44	0.74
	T4	4.70	2,541	0.82	0.46
Residue	T1	2.27	4,985	0.11	0.18
	T4	2.52	4,014	0.18	0.31
	Sx	0.15	447	0.09	0.14

T1 and T4 are treatments with and without organic matter from season 2.

Seed and residue yields of mung bean in season 4 were also increased by the incorporation of organic matter. A comparison of yields in season 2 and 4 indicated the benefits of continued supply of organic matter in increasing yields of mung. This clearly shows the importance of adding organic matter to sustain yields and to enhance productivity of tropical crops. Again, the N derived from  $^{15}\text{N}$  fertilizer supplied 3 seasons earlier was present in small quantities. The  $^{15}\text{N}$  derived from the applied fertilizer was again higher in seeds of mung bean in T1, where crop residues were added. In contrast, the Ndff in both treatments was similar in the residue. This again suggests that the addition of organic matter enhanced the availability of applied N, especially that left after cropping. This N derived from the applied fertilizer was assimilated more by seed than by the residue.

Nitrogen and organic matter contents have a significant impact on the sustainability of tropical soils and are of importance in modern smallholder production units in these regions. The results of this study clearly illustrate that the application of organic matter in the form of crop residues, which are otherwise either burnt or discarded, could have significant impact on the retention of applied N fertilizer. This, in turn, would increase productivity of subsequent crops. Thus, farmers should be encouraged to apply residues after harvest to retain added N fertilizer, which is easily leached from the soil. This process would then increase productivity, improve sustainability, and bring a better return to the investment made by the smallholder farmer.

#### ACKNOWLEDGEMENTS

This research was carried out as a part of a Coordinated Research Project on the Management of Organic Matter and Nutrient Turnover for Increased Sustainable Production and Environmental Protection (SRL 9038). Gratitude is expressed to Dr. D.G. Keerthisinghe of the IAEA for his guidance and to the IAEA for funds and support granted to this project.

## REFERENCES

- [1] FAO, Agricultural Research Priorities in the Asia Pacific region, An APAARI Overview, FAO Regional Office, Bangkok.
- [2] KUMAR, V., et al., Recycling of crop wastes and green manure and their impact on yield and nutrient uptake of wetland rice, *J. Agric. Sci.* **132** (1999) 149–154.
- [3] SWIFT, M.J., WOOMER, P., “Organic matter and the sustainability of agricultural systems”, *Soil Organic Matter Dynamics and Sustainability of Tropical Agriculture* (MULONGOY, K., MERCKX, R., Eds.), Wiley and Sons, New York (1993) 3–18.

## USE OF $^{14}\text{C}$ TO ASSESS THE AGE OF HUMIC SUBSTANCES

M.A. ANISIMOVA, O.A. CHICHAGOVA  
Russian Academy of Science,  
Moscow, Russian Federation

E. SCHNUG  
Agricultural Research Centre (FAL),  
Braunschweig, Germany

The radiocarbon method was developed in the post-war years by a team at the University of Chicago, led by the late Professor Willard F. Libby. It has been used in many fields of endeavour, including palaeoclimatology, archaeology, geology, hydrology, biogeochemistry, and palaeopedology to determine age of various materials. Carbon-14 analyses of soil organic matter (SOM) in burial soil can be used for paleoclimatic and paleogeographical reconstructions, because the composition and the properties of the SOM reflect the trend and intensity of soil-formation processes. Isolated from the biologically active medium neither the composition of humus nor the properties of SOM of paleosols are affected by secondary alteration [1]. Also, attempts to establish the age of modern soils are rendered inaccurate by exchanges of soil carbon with atmospheric  $\text{CO}_2$ ; only rate of soil carbon renovation can be determined.

Formerly, in order to define renovation rate of organic carbon in soils the coefficient of renovation ( $K_r$ ) was used, i.e. the integral index of organic carbon renovation as a result both of biochemical reactions of mineralization and of migration in the soil profile [2].

The use of  $^{14}\text{C}$  techniques to determine the age of burial soil, or to determine the renovation rate of carbon in modern soils, generally includes the following steps:

- pretreatment of soil material with 10% HCl to remove absorbed carbonates,
- boiling of sample in 5% NaOH to produce two main fractions of soil organic matter: humic acids (acid-insoluble) and fulvic acids (acid-soluble),
- carbonization of extracted organic material,
- interaction of coal with molten lithium to form lithium carbide ( $\text{Li}_2\text{C}_2$ ),
- hydrolyzation to acetylene ( $\text{C}_2\text{H}_2$ ),
- catalytical trimerization to benzene ( $\text{C}_6\text{H}_6$ ),
- measurement of the activity of  $^{14}\text{C}$  in benzene, and
- calculation of radiocarbon age of the sample.

In order to determine radiocarbon age (or to define the carbon renovation rate) in a soil sample, it is necessary to obtain the amount of radiocarbon. This is done by measuring the radioactivity of the sample (the conventional beta-counting method) or by directly counting the radiocarbon atoms using the accelerator mass spectrometry (AMS) method. In AMS, the radiocarbon atoms are detected directly, instead of waiting for them to decay.

A scheme for AMS action is shown on the Fig. 1.

The main advantage of AMS over conventional beta-counting is its much greater sensitivity. In common with other kinds of mass spectrometry, it is performed by converting the atoms in the sample into a beam of fast-moving ions, the mass of which is then measured by the application of magnetic and electric fields.

The application of  $^{14}\text{C}$  dating of recent and fossil soils allows the elucidation of soil genesis and evolution, humus formation, and organic matter metamorphosis [3]. Furthermore, the use of the  $^{14}\text{C}$  technique to determine the age of the oldest fractions of soil organic matter can provide more-precise information on the time of formation of modern soils.

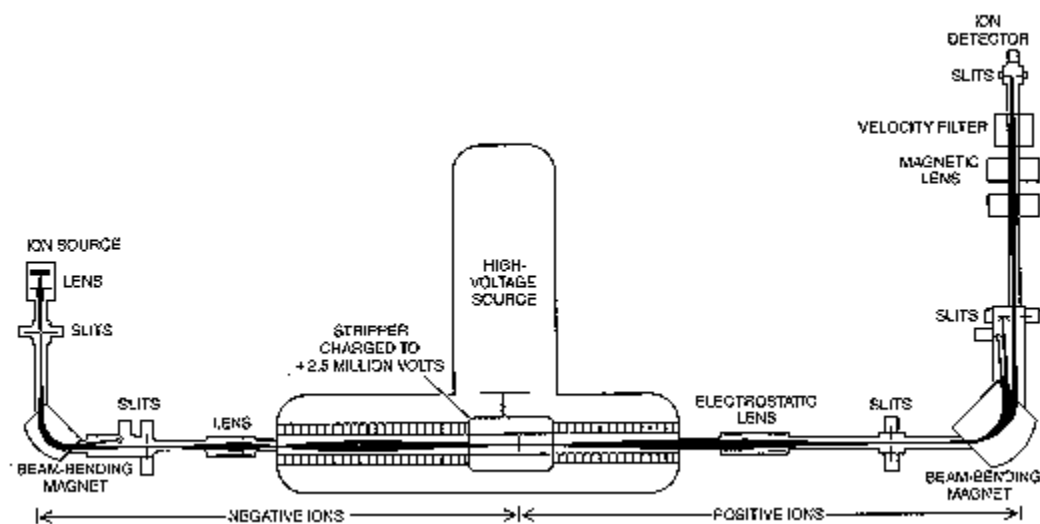


FIG. 1. A schematic diagram of accelerator mass spectrometry (AMS).

## REFERENCES

- [1] CHICHAGOVA, O.A., Composition, properties and radiocarbon age of humus in paleosols, *GeoJournal* **36** (1995) 208.
- [2] CHERKINSKIY, A.E., BROVKIN, V.A., Dynamics of radiocarbon in soils, *Radiocarbon* **35** (1993) 363–367.
- [3] CHICHAGOVA, O.A., CHERKINSKIY, A.E., Problems in radiocarbon dating of soils, *Radiocarbon* **35** (1993) 351.



# MOVEMENT OF CARBON TO ROOTS OF FOOD LEGUMES AS AFFECTED BY SOIL MOISTURE AND FERTILIZER POTASSIUM

U.R. SANGAKKARA  
University of Peradeniya,  
Peradeniya, Sri Lanka

M. FREHNER, J. NOSBERGER  
Institute of Plant Sciences,  
Zurich, Switzerland

Food legumes are an important component of agricultural systems in developing countries [1]. They are cultivated in a wide range of environments where soil moisture could be generally considered limiting for optimum growth and yield [2]. In terms of vegetative growth, root systems of plants adapt to extract the limited moisture from dry soils. Although it is known that fertilizer K helps plants mitigate soil moisture stress [3,4], this has not been evaluated on a comparative basis in food legumes. Therefore, a study was carried out under controlled conditions to determine the role of  $K^+$  in the movement of labelled C in three tropical food legumes that have differing optimal soil-moisture requirements. Common bean (*Phaseolus vulgaris*), mung bean (*Vigna radiata*), and cowpea (*Vigna unguiculata*) were grown under two moisture regimes with three levels of  $K^+$  (0.1, 1.0 and 3.0 mM K).

Uniform seedlings of the three species were transplanted into pots containing quartz sand, maintained at two soil-moisture levels (High, less than 25% depleted of available soil moisture; Low, more than 50% depleted), by weighing at 3-day intervals and adding the required water or nutrient solution. The modified nutrient solutions had 0.1, 1.0 or 3.0 mM  $K^+$  with 1.5 mM N.

The plants were kept in a growth chamber, maintained at 25°/18°C day/night, with a 16-h photoperiod, and at 60% humidity. At the V4 growth stage of each species, four plants were selected randomly, and approximately 1 cm<sup>2</sup> of the upper surface of the youngest fully expanded leaf abraded with Carborundum. Thereafter, 5 µL of <sup>14</sup>C contained in a 5-mM solution of sucrose (148 KBq) was applied to the abraded area. Another 10 µL of unlabelled sucrose was applied to the same area 10 min later and the plants replaced. After 24 h, they were carefully removed, roots washed, dried and weighed. Thereafter, the samples were ground and digested, and labelled C determined by liquid scintillation.

Cowpea, the species most adapted to soil-moisture stress, in general had the highest root weights. The root weights of mung bean, which requires moderate soil moisture, were generally intermediate, and common bean, the least drought-tolerant, had the lowest values for root dry weight (Table I).

TABLE I. ROOT DRY WEIGHTS AS AFFECTED BY MOISTURE AND K

Species	Soil moisture	Root dry weight (mg/plant)		
		0.1 mM K	1.0 mM K	3.0 mM K
Common bean	High	104	145	169
	Low	165	171	211
	Sx	13.4	20.8	9.7
Mung bean	High	116	167	230
	Low	194	210	224
	Sx	41.2	18.0	28.6
Cowpea	High	149	178	229
	Low	192	221	301
	Sx	22.9	34.1	40.5
CV%		10.7	8.5	15.9

TABLE II. PARTITIONING OF  $^{14}\text{C}$  TO ROOTS IN RELATION TO MOISTURE AND K

Species	Soil moisture	$^{14}\text{C}$ counts in roots (Bq/s)		
		0.1 mM K	1.0 mM K	3.0 mM K
Common bean	High	861(0.58) <sup>a</sup>	1,064(1.5)	6,512(4.5)
	Low	110(0.07)	957(0.64)	3,356(2.3)
	Sx	14.4	35.7	88.4
Mung bean	High	85(0.57)	2,160(1.5)	3,464(2.3)
	Low	877(0.59)	2,430(1.6)	6,834(4.6)
	Sx	21.7	210	1,544
Cowpea	High	1,151(0.77)	1,677(1.1)	3,979(2.7)
	Low	601(0.40)	2,874(1.9)	3,167(2.1)
	Sx	199	351	582

<sup>a</sup>Percentage of  $^{14}\text{C}$  in roots in relation in that supplied to the leaf.

All plants grown under the lower soil moisture regime had heavier roots (Table I), due to extensive branching (data not presented).

Added K increased root dry weights. The most significant affect was in cowpea, grown under low soil moisture: 3.0 mM K increased root dry weight by 77% and 51% under low and high soil moisture, respectively, when compared to values at 0.1 mM K. In mung bean, increases were 32 were 34%. In contrast, in common bean, the impact of 3.0 mM K was greater at the higher soil moisture level (58%) than at the lower (47%). The reasons for these observations become evident from the  $^{14}\text{C}$  data.

The movement of  $^{14}\text{C}$  into roots was higher under the lower soil moisture regime in cowpea and mung bean (Table II). This phenomenon is very clear at 0.1 and 1.0 mM K in these species (Table II). In contrast, the movement of  $^{14}\text{C}$  was greater at the higher soil moisture level in common bean. The movement of  $^{14}\text{C}$  into roots was consistently enhanced by K. In cowpea, the most significant increment in  $^{14}\text{C}$  movement was with the supply of 1.0 mM K, while in mung bean it was with 3.0 mM K, both at lower soil moisture. In common bean, this phenomenon was most evident with higher soil moisture with 3.0 mM K.

This study elucidates the impact of K in mitigating soil-moisture stress in three food legumes that are consumed extensively in the tropics. Potassium helped develop the root system by enhancing C movement from leaves, thus enabling extraction of soil moisture. This phenomenon was most evident in the more drought-resistant species, cowpea and mung bean, but K also helped common bean to develop its root system with higher soil moisture.

#### ACKNOWLEDGEMENTS

Gratitude is expressed to ETH Zurich for funding and to Mr. K Girgenrath for assistance with  $^{14}\text{C}$  analysis.

#### REFERENCES

- [1] FAO, Year Book, Food and Agriculture Organization, Rome (1998) 207.
- [2] CHAUHAN, Y.S., et al., Adaptation of extra short duration pigeon pea in the short rainy season of a tropical bimodal rainfall environment, *Exp. Agric.* **35** (1999) 87–100.
- [3] MARSCHNER, H., Mineral Nutrition of Higher Plants, Academic Press, London (1996).
- [4] SANGAKKARA, U.R., et al., Response of root branching and shoot water potentials of frenchbeans (*Phaseolus vulgaris* L.) to soil moisture and fertilizer potassium, *J. Agron. Crop Sci.* **177** (1996) 165–173.

## ASSESSMENT OF PHOSPHORUS AVAILABILITY IN LONG TERM FIELD EXPERIMENTS TESTING P-INPUT REGIMES

A. GALLET, S. SINAJ, E. FROSSARD  
Institute of Plant Sciences, Lindau

R. FLISCH  
Swiss Federal Research Station for Agroecology, Zurich

Switzerland

Most agricultural soils in Switzerland have large reserves of P in the upper horizons. Excessive P content can lead to losses to surface and ground waters and to eutrophication. Long term field experiments are aimed at determining the optimum level of soil available P for sustainable crop production. The objective of this work was to measure, using three analytical methods, the changes in P availability of soil samples from two long-term field experiments, testing different P-input regimes in relation with crop yields and P uptake.

Two field trials with crop rotations, established in 1989 at Cadenazzo (canton of Ticino, Switzerland) and Ellighausen (canton of Thurgau, Switzerland) were studied. The trials include the following three superphosphate treatments: control (no P applied since 1989); P<sub>norm</sub> (P input approximately the same as P output by the crops) and 5/3 P<sub>norm</sub>. Nitrogen and K are applied also according to the norm concept (input approximately equal to output in the harvested crop).

Samples of topsoil (0–20 cm) from each of four replicates in 1989, 1993, and 1998 were analysed for soil P availability as follows: extraction by CO<sub>2</sub>-saturated water [1], extraction by acetate-NH<sub>4</sub>-EDTA mixture [2], and the isotopic exchange kinetics method [3].

Results from the isotopic exchange kinetics method were analysed by a pluricompartimental model, which quantifies the phosphate ions present in compartments of differing mobility: the pool of P isotopically exchangeable within 1 min (the quantity of P immediately available to roots, E<sub>1min</sub>); the pool of P isotopically exchangeable between 1 min and 24 h (E<sub>1min-24h</sub>); the pool of P isotopically exchangeable between 24 h and 3 months (E<sub>24h-3m</sub>) and the pool of P that cannot be isotopically exchanged within 3 months (E<sub>>3months</sub>). The amounts of P present in these pools were calculated by the method proposed by Fardeau [3]. The amounts of P exported by the harvested parts of the crops were measured in order to calculate P balances.

Only the results of the control-0P and 5/3 P<sub>norm</sub> treatment for the Cadenazzo field trial are reported.

After 10 years of field trial, the results show that the P balance (Fig. 1) in the 0P treatment was negative (–88 mg P kg soil<sup>–1</sup>) and positive for the 5/3 P<sub>norm</sub> treatment (+70 mg P kg soil<sup>–1</sup>). Yields (data not shown) and P uptake (Fig. 2) were the same for both treatments despite these important differences in P balance. The high P uptake in both treatments was explained by the high concentrations of P in the pool of free ions (E<sub>1min</sub>)—observed for the duration of the trial—well above the critical limit of 3 to 4 mg P kg soil<sup>–1</sup> reported for winter wheat [4].

The quantity of P present in the pool of free ions decreased in both treatments with time (Table I), although more strongly in the 0P treatment than in the 5/3P<sub>norm</sub> treatment. In the 0P treatment, E<sub>>3months</sub> decreased significantly and the P mobilized from this pool was redistributed in the pools of P exchangeable in the medium term (E<sub>1min-24h</sub>, E<sub>24h-3m</sub>). In the 5/3 P<sub>norm</sub> treatment, an excess of 60 mg P kg soil<sup>–1</sup> added in a water-soluble form was redistributed into the pools of P exchangeable in the medium term (E<sub>1min-24h</sub>, E<sub>24h-3m</sub>).

As observed for E<sub>1min</sub> values, the quantities of P extracted by CO<sub>2</sub>-saturated water and by the acetate-NH<sub>4</sub>-EDTA mixture decreased with time, both in the 0P and in the 5/3P<sub>norm</sub> treatments (Figs 3 and 4).

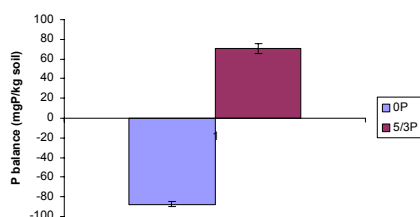


FIG. 1. Cadenazzo P balance in 1998.

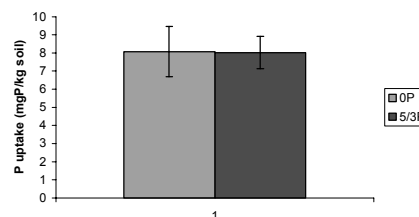


FIG. 2. P uptake by wheat in 1998 at Cadenazzo.

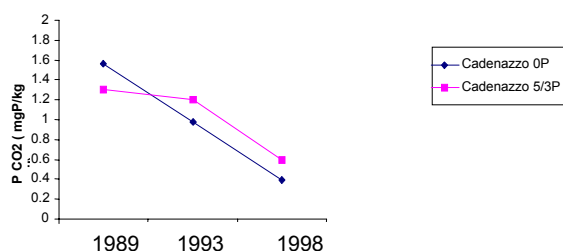


FIG. 3. Extractable P, the Dirks-Scheffer method.

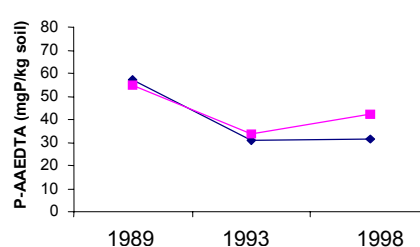


FIG. 4. Extractable P, the Cottenie method.

TABLE 1. ISOTOPICALLY EXCHANGEABLE P  
IN 1989 AND 1998 FOR THE 0P AND 5/3P  
TREATMENTS AT CADENAZZO

Treatment	Year	$E_{1\text{min}}$	$E_{1\text{min-24h}}$	$E_{24\text{h-3m}}$	$E_{>3\text{months}}$
		(mg P/kg soil)			
0P	1989	11	44	87	808
	1998	5	47	135	722
5/3P	1989	12	43	79	822
	1998	9	54	129	804

Ten years without fertilization affected neither plant yield nor P uptake. The isotopic exchange kinetic method is useful for correctly assessing the soil P availability and for predicting the efficiency of P fertilizers as they affect crop yields over years. Results obtained with the other two extraction methods showed the same trends with time as those observed in terms of the amount of P isotopically exchangeable within 1 min.

## REFERENCES

- [1] DIRKS-SCHEFFER, Der Kohlensäure-Bikarbonatauszug und der Wasserauszug als Grundlage zur Ermittlung der Phosphorsäurebedürftigkeit der Böden, *Landwirtschaftl. Jahrbücher* **71** (1930) 73–99.
- [2] COTTENIE, A., *Chemical Analysis of Plant and Soils*, Laboratory of Analytical and Agrochemistry State University Ghent, Ghent (1982).
- [3] FARDEAU, J.C., Le phosphore assimilable des sols: sa représentation par un modèle fonctionnel à plusieurs compartiments, *Agronomie* **13** (1993) 1–15.
- [4] MOREL, C., et al., 1992. La fertilisation phosphatée de la culture du blé, *Agronomie* **12** (1992) 565–579.

## NITROGEN FIXATION BY VEGETABLE AND GREEN-MANURE LEGUMES AS INFLUENCED BY INTERCROPPING WITH MAIZE

M.K. SCHNEIDER, W. RICHNER, P. STAMP,  
Institute of Plant Science,  
Zurich, Switzerland

U.R. SANGAKKARA  
University of Peradeniya,  
Peradeniya, Sri Lanka

The fixation of atmospheric N<sub>2</sub> by two legumes was assessed in a field experiment on an alfisol in the semi-dry midlands of Sri Lanka. Our overall objective was to determine the effects of green manuring in tropical rain-fed maize (*Zea mays* L.) cropping systems.

*Crotalaria juncea* L. was grown as a monocrop and intercropped with maize (cv. Ruwan), and *Phaseolus vulgaris* L. (cv. RIK-692) was also intercropped with maize. The trial was carried out in a randomized complete block design at three sites, at each of which were two replications. In the monocrop of *C. juncea*, 29.7 plants/m<sup>2</sup> were established in 30-cm rows. In the intercrops, 13.9 plants/m<sup>2</sup> of *C. juncea* and 18.6 plants/m<sup>2</sup> of *P. vulgaris* were established between the rows of maize (5.5 plants/m<sup>2</sup> in 60-cm rows).

To the rows of maize, 25 kg N/ha were applied as basal dressing and 45 kg N/ha as top dressing. Twenty kg N/ha were applied to monocropped *C. juncea*. Fertilization with P and K was as recommended for maize. In microplots of 1 m<sup>2</sup>, 20 kg N/ha of the basal dressing were substituted by an equal amount of <sup>15</sup>N labelled ammonium sulphate (10% enrichment). *Phaseolus vulgaris* was sampled at flowering (5 weeks after sowing) and maize at silking (10 weeks after sowing). The samples were dried and the total N and <sup>15</sup>N contents were determined. The proportion of N derived from fixation was calculated from the formula:

$$\%N \text{ from fixation} = \left[ 1 - \frac{(^{15}\text{N}_{\text{excess}})_{\text{legume}}}{(^{15}\text{N}_{\text{excess}})_{\text{reference}}} \right] \times 100$$

The intercropped maize was used as the non-fixing reference. Biomass production of the crops was determined at 10 weeks after sowing.

Five weeks after sowing, N contents and %N from fixation were in the order: intercropped *C. juncea* > monocropped *C. juncea* > intercropped *P. vulgaris* (Table I). Ten weeks after sowing, N contents, %N from fixation and amount fixed were in the order: monocropped *C. juncea* > intercropped *C. juncea* > intercropped *P. vulgaris*. *Phaseolus vulgaris* had the highest per-plant weight and monocropped *C. juncea* produced the greatest biomass per unit area (Table II).

Our study confirmed that the green-manure legume *C. juncea* fixes considerably more N than does the vegetable legume *P. vulgaris*. However, the N<sub>2</sub> fixation of both species was comparatively low. This may be attributed to the N applied to maize, which was to some extent also available to the intercropped legumes. Higher N<sub>2</sub> fixation rates have been reported in *P. vulgaris* in studies with less or no N fertilization [1,2]. This explains also the differences between monocropped and intercropped *C. juncea*. At an early stage, competition from maize enhanced the N<sub>2</sub> fixation of the intercropped legumes. Later, as a result of ageing and of the top dressing to maize, *P. vulgaris* fixed very little N.

The N<sub>2</sub> fixation of intercropped *C. juncea* was the same at both stages, whereas it increased at the later stage when monocropped. Lack of data on the effects of low to moderate N fertilization on N<sub>2</sub> fixation was recently emphasized [3]. However, drought and scarcity of nutrients other than N may also have affected N<sub>2</sub> fixation.

TABLE I. INFLUENCE OF INTERCROPPED MAIZE ON SHOOT N AND N<sub>2</sub> FIXATION IN *Crotalaria juncea* AND *Phaseolus vulgaris* AT 5 WEEKS AFTER SOWING)

Legume	N content (mg/g)	Ndfa <sup>a</sup> (%)
Monocropped <i>C. juncea</i>	35.0a <sup>b</sup>	21%a
Intercropped <i>C. juncea</i>	39.6a	26%a
Intercropped <i>P. vulgaris</i>	25.6b	18%a

<sup>a</sup>N derived from the atmosphere determined using the <sup>15</sup>N-dilution method.

<sup>b</sup>Mean separation in columns by Fisher's LSD test ( $P < 0.05$ ).

TABLE II. INFLUENCE OF INTERCROPPED MAIZE ON SHOOT N CONTENTS, N<sub>2</sub> FIXATION AND BIOMASS PRODUCTION OF *Crotalaria juncea* AND *Phaseolus vulgaris* AT 10 WEEKS AFTER SOWING

Legume	N content (mg/g)	Ndfa <sup>a</sup> (%)	Fixed N (mg/plant)	Fixed N (mg/m <sup>2</sup> )	Biomass (g/plant)	Biomass (g/m <sup>2</sup> )
Monocropped <i>C. juncea</i>	27.0a <sup>b</sup>	40%a	64.6a	2480a	5.4ab	211a
Intercropped <i>C. juncea</i>	26.8a	25%ab	37.6ab	686b	4.8a	82b
Intercropped <i>P. vulgaris</i>	18.2b	10%b	9.7b	233b	6.5b <sup>c</sup>	116b <sup>c</sup>

<sup>a</sup>N derived from the atmosphere determined using the <sup>15</sup>N-dilution method.

<sup>b</sup>Mean separation in columns by Fisher's LSD test ( $P < 0.05$ ).

<sup>c</sup>Biomass of intercropped *P. vulgaris* includes the harvested green pods.

Intercropping with maize reduced the biomass of *C. juncea* to a greater extent than that expected as a consequence of plant density. Nitrogen fixed by intercropped *C. juncea* was only 27% of that of the monocrop. Field trials showed that monocropping green manure legumes may be more productive than intercropping them with food crops [4]. We conclude that green manure legumes may potentially bring more N into tropical maize cropping systems when monocropped than when intercropped.

## REFERENCES

- [1] GILLER, K.E., et al., Nitrogen transfer from *Phaseolus* bean to intercropped maize measured using N-15-enrichment and N-15-isotope dilution methods, *Soil Biol. Bioch.* **23** (1991) 339–346.
- [2] SANGAKKARA, U.R., et al., Soil moisture and potassium affect the performance of symbiotic nitrogen fixation in faba bean and common bean, *Plant Soil* **184** (1996) 123–130.
- [3] CHALK, P.M., LADHA, J.K., Estimation of legume symbiotic dependence: an evaluation of techniques based on N-15 dilution, *Soil Biol. Biochem.* **31** (1999) 1901–1917.
- [4] FISCHLER, M., et al., *Crotalaria* (*C. ochroleuca* G. Don.) as a green manure in maize-bean cropping systems in Uganda, *Field Crops Res.* **61** (1999) 97–107.

## DRIP FERTIGATION FOR IMPROVEMENT OF COTTON YIELD, NITROGEN RECOVERY AND WATER USE EFFICIENCY

M. JANAT

Atomic Energy Commission,  
Damascus, Syrian Arab Republic

Drip-fertigation experiments with cotton were carried out in 1999 at the research station to confirm a previous study and in a farmer's field to test the efficacy of this relatively new irrigation technology. Two irrigation methods, surface and drip fertigation, with five N rates; (N1=60, N2=120, N3= 180, N4=240, N5=300 kg N/ ha) were used at both locations. Nitrogen fertilizer, as urea, was broadcast for the surface-irrigated cotton in four unequally split applications, as locally recommended. Also, N fertilizer, as urea, was injected through the drip system in eight equal applications. Labelled  $^{15}\text{N}$  subplots were established for both drip-fertigated and surface-irrigated treatments. The neutron probe and tensionics were used to monitor soil-moisture status, nitrate movement, and to provide feedback data for irrigation scheduling. Treatments were arranged in a randomized block design with six replicates; this set-up allowed simultaneous comparison of fertigation and surface irrigation. The neutron probe was employed to precisely determine when to irrigate and establish an irrigation schedule for drip fertigation technique. And  $^{15}\text{N}$ -enriched urea permitted determination and comparison of fertilizer-N uptake [1] by the cotton under the two irrigation methods.

Results revealed savings in irrigation water due to the employment of drip fertigation, in excess of 30% at the farmer level and 37% at the research station level compared to surface irrigation. Field water-use efficiency ( $E_f$ ) was significantly improved with drip fertigation. The magnitude of the improvement exceeded 90% in most cases (Fig. 1). This result indicates that a large amount of surface-irrigation water was applied regardless of need of the cotton at both locations, and with good management such wasted water could be saved. The increases in cotton-seed yield at the end-user level for the drip-fertigated treatments were 55, 0, 32, and 43% in comparison with the corresponding surface-irrigated treatments N1, N2, N3, and N4 respectively (data not shown). On the other hand, at the research station, cotton-seed yields of the fertigated-treatments were increased by 38, 22, 30, 32, and 31% relative to the corresponding surface-irrigated treatments, while values for N recovery by both drip-fertigated and surface-irrigated cotton were lower than expected (Table I). We observed this phenomenon in previous years; it is related to the lateral movement of  $^{15}\text{NO}_3^-$  and  $^{14}\text{NO}_3^-$  from the labelled subplots to adjacent unlabelled subplots and vice-versa. Field germination percentage (FGP) of cottonseeds was significantly increased by adoption of drip fertigation. Increases in FGP exceeded 40% in some cases (Table II).

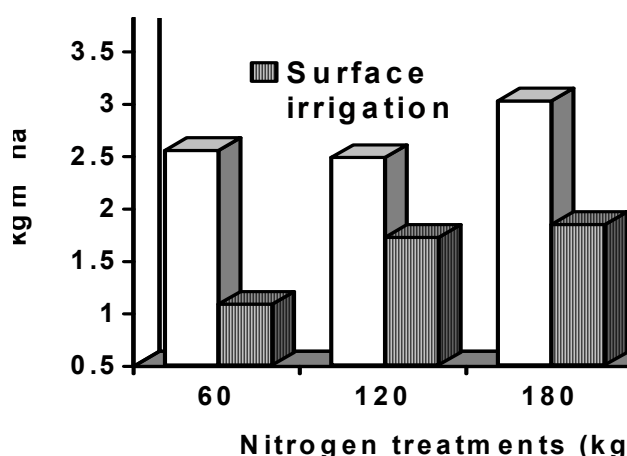


FIG. 1. Water use efficiency by cotton in relation to irrigation method and N level.

TABLE I. IRRIGATION METHOD AND N-RATE EFFECTS ON COTTON YIELD, EARLINESS AND RECOVERY OF FERTILIZER N

	N1	N2	N3	N4	N5
	(kg ha <sup>-1</sup> )				
Fertigation					
1 <sup>st</sup> picking	2,596a	2,711a	2,553a	2,298a	2,674a
2 <sup>nd</sup> picking	1,133a	1,239a	1,208a	1,257a	1,365a
Total yield	3,729a	3,950a	3,761a	3,555a	4,037a
Earliness (%)	70a	69a	67a	65a	66a
N recovery (%)	35	50	35	41	26
Surface					
1 <sup>st</sup> picking	1,644a <sup>a</sup>	1,463a	1,531a	1,536a	1,550a
2 <sup>nd</sup> picking	1,043a	1,771a	1,363a	1,044a	1,544a
Total yield	2,696a	3,241a	2,893a	2,696a	3,094a
Earliness (%)	61a	47a	54a	58a	52a
N recovery (%)	36	32	25	22	21

<sup>a</sup>Means followed by the same letter within a row are not statistically different at  $P = 0.05$  by DMRT.

TABLE II. IRRIGATION METHOD AND N-RATE EFFECTS ON FIELD GERMINATION PERCENTAGE OF COTTON

Location/Irrigation method	N1	N2	N3	N4	N5
	(%)				
Hama					
Fertigation	88a <sup>a</sup>	92a	89a	85a	
Surface irrigation	68a	68a	70a	67a	
Der EL Hajar					
Fertigation	92a	92a	85a	88a	80a
Surface irrigation	57a	51a	52a	59a	51a

<sup>a</sup>Means followed by the same letter within a row are not statistically different at  $P = 0.05$  by DMRT.

## ACKNOWLEDGEMENTS

The author thanks the SAEC and IAEA for their valued support. This project is part of an IAEA TC regional project (RAW/5/007) funded by the TC section of the IAEA and the SAEC.

## REFERENCE

- [1] ZAPATA, F., "Isotope techniques in soil fertility and plant nutrition studies", Use of Nuclear Techniques in Studies of Soil-Plant Relationships, Series No. 2 (HARDARSON, G., Ed.), IAEA, Vienna (1990).



# EFFECTS OF PHOSPHORUS NUTRITION ON GROWTH AND RADIOPHOSPHORUS UPTAKE IN *Sorghum bicolor* GENOTYPES

R. CAMACHO

University Rómulo Gallegos,  
San Juan de los Morros, Venezuela

E. MALAVOLTA

University of Sao Paulo,  
Piracicaba, Brazil

Seeds of eight genotypes of grain sorghum (Criollo-1, Criollo-8, Sefloarca-7, Sefloarca-10, Himeca-101, Himeca-303, Pioneer YSB-83, and Wac 8228-Br) were germinated in vermiculite moistened with a  $10^{-4}$  M solution of calcium sulphate. Seedlings were kept for 10 days in the solution of Johnson et al. [1] diluted five-fold. Afterwards, the plants were transferred to the full strength solution modified in order to supply three P concentrations, 0, 0.5, and 1.0 mM, wherein they were grown for 3 weeks, when growth, leaf P and uptake of  $^{32}\text{P}$  were evaluated.

Immediately after harvesting, the uptake of  $^{32}\text{P}$  was assayed by the excised-root method of Harrison and Helliwell [2]. The roots were washed three times with distilled water, dried at 70°C, and a nitric ( $\text{HNO}_3$ )-perchloric ( $\text{HClO}_4$ ) acids extracts was prepared according to Malavolta et al. [3]. Total P was analyzed by the metavanadate method [3].

Table I shows the effects of P on dry-matter production. Except for Criollo-8, increasing P in the nutrient solution decreased the root dry matter (RDM), but had the opposite effect on shoot dry matter (SDM) and total dry matter (TDM), as expected. Himeca-101 showed consistently high root, shoot and total dry matter yields in the -P treatment.

These data verify preliminary findings, confirming that Himeca-101 can benefit from low concentrations of P in the growth substrate [4]. On the other hand, there were no variations in the dry matter among genotypes at the highest level of P. For all genotypes, except Sefloarca-10, increasing P concentrations above 5 mM had negative effects on shoot DM.

TABLE I. EFFECT OF THREE P LEVELS (mM) ON ROOT, SHOOT AND TOTAL DRY MATTER YIELDS OF EIGHT GRAIN SORGHUM GENOTYPES

Hybrid	RDM				SDM				TDM			
	0	0.5	1.0	sig	0	0.5	1.0	sig	0	0.5	1.0	sig
(g plant <sup>-1</sup> )												
C-1 <sup>a</sup>	0.57b <sup>b</sup>	0.39b	0.42a	** <sup>c</sup>	0.90b	2.28a	1.89a	**	1.47b	2.65b	2.31a	**
C-8	0.37c	0.57b	0.40a	**	0.77c	2.32a	2.04a	**	1.14c	2.89a	2.44a	**
S-7	0.59b	0.41b	0.29a	**	1.48a	2.19a	1.73a	**	2.07a	2.60b	2.01a	**
S-10	0.45b	0.33c	0.32a	*	0.96b	1.79b	1.86a	**	1.41b	2.12b	2.18a	**
H-101	0.92a	0.81a	0.46a	**	1.88a	2.77a	1.91ab	**	2.79a	3.58a	2.37a	**
H-303	0.64b	0.43b	0.39a	**	1.34a	1.98b	1.71a	**	1.98a	2.42b	2.10a	NS
P	0.42b	0.27d	0.24a	**	1.05b	1.68b	1.50a	**	1.46b	1.98c	1.74a	NS
W	0.60b	0.52b	0.47a	**	1.37a	2.03b	1.87a	**	1.97a	2.55b	2.33a	**

<sup>a</sup>C, Criollo; S, Sefloarca; H, Himeca; P, Pioneer YSB-83; W, Wac 8228-Br.

<sup>b</sup>Values within a column followed by the same letter are not significantly different ( $P < 0.05$ ) according to Tukey's test.

<sup>c</sup>\* and \*\* indicate significance at the 0.05 and 0.01 probability levels, respectively; NS: no significant.

TABLE II EFFECT OF THREE P LEVELS (mM ) ON TOTAL LEAF P AND UPTAKE OF  $^{32}\text{P}$  IN EIGHT GRAIN SORGHUM GENOTYPES

Genotype	Total P (g kg <sup>-1</sup> )				$^{32}\text{P}$ (μmol g <sup>-1</sup> RDM 15 min <sup>-1</sup> )			
	0	0.5	1.0	Sig	0	0.5	1.0	Sig
C-1 <sup>a</sup>	1.90a <sup>b</sup>	14.6a	14.2b	***	0.62bc	0.19b	0.17b	**
C-2	1.63a	15.8a	17.8a	**	0.83ab	0.14b	0.13b	**
S-7	2.47a	14.3a	13.2b	**	0.84ab	0.32ab	0.41ab	**
S-10	1.93a	12.7b	12.4c	**	0.88ab	0.26ab	0.24ab	**
H-101	2.07a	15.8a	14.7b	**	0.25c	0.08b	0.07b	**
H-303	2.03a	15.1a	15.6a	**	0.63bc	0.22b	0.20b	**
P	2.07a	13.9a	13.5b	**	1.08a	0.61a	0.51a	**
W	2.00a	15.30a	14.6b	**	0.97bc	0.21b	0.19b	**

<sup>a</sup>C, Criollo; S, Sefloarca; H, Himeca; P, Pioneer YSB-83; W, Wac 8228-Br.

<sup>b</sup>Values within a column followed by the same letter are not significantly different ( $P < 0.05$ ) according to Tukey's test.

<sup>c</sup>\*\*Significance at the 0.01 probability level; NS: no significant.

There were no variations in foliar P concentrations when P was not added; differences among genotypes were greatest at the highest solution P (Table II). It has been reported elsewhere that variations in foliar P are least when substrate P is low and greatest when substrate P is high. On the other hand, leaf P was strongly enhanced by increasing solution P up to 0.5 mM, with little variation among genotypes, whereas enhancing P from 0.5 to 1.0 mM had negative effects in six hybrids (Table II). Since, in our work, increased P in the nutrient solution from 0.5 to 1.0 mM was accompanied by decreased TDM (Table I) and leaf P (Table II), we suspect toxic effects of P.

As expected, radiophosphorus uptake from the test solution by excised roots was highest in the absence of P, and an increase of P level in the culture solution decreased P influx (Table II). These results confirm our preliminary findings.

Irrespective of P level in the nutrient solution, Himeca-101 showed the lowest uptake rates of  $^{32}\text{P}$  (Table II) and one of the highest yields of dry matter (Table I), while Pioneer showed the opposite effect. In Himeca-101, P uptake remained very low, at about 0.08 mol g<sup>-1</sup> DM 15 min<sup>-1</sup>, in the range from 0.5 to 1.0 mM. This indicates that Himeca-101 has the capacity to stop or to reduce P uptake when grown at elevated concentrations of P, thus avoiding toxic effects.

## REFERENCES

- [1] JOHNSON, C.M., et al., Comparative chlorine requirement of different plants species, *Plant Soil* **8** (1957) 337–353.
- [2] HARRISON, A.F., HELLIWELL, D.R., A bioassay for comparing phosphorus availability in soils, *J. Appl. Ecol.* **16** (1979) 497–505.
- [3] MALAVOLTA, E., et al., *Avaliação do Estado Nutricional das Plantas: Princípios e Aplicações*, 2 Ed., Associação Brasileira para Pesquisa da Potassa e do Fósforo, Piracicaba (1997).
- [4] CAMACHO, R., MALAVOLTA, E., Effect of phosphorus supply on growth and acid phosphatase activity in grain sorghum hybrids, *Phyton* **66** (2000) (in press).

## USE OF LABELLED PLANT RESIDUES TO STUDY THE IMPACT OF RESIDUE INCORPORATION ON SOIL CARBON AND AGGREGATION

N. BLAIR, A.R. TILL, R.D. FAULKNER  
University of New England,  
Armidale

K.E. PRINCE  
Australian Nuclear and Scientific Technology Organisation,  
Menai

New South Wales, Australia

Soil structural and soil organic matter decline is a worldwide problem and the return of plant residues is being used in many areas to attempt to increase soil C, which can lead to an improvement in structure. Studying the impact of residue incorporation on soil C can be difficult because of the large background of residual C. The use of isotope-labelled plant material allows the investigation of the effects of newly incorporated residue on C dynamics. An experiment was conducted to evaluate the effect of plant residues—with differing breakdown rates, incubated at different temperatures and for various time periods—on the incorporation of C and the stability of the soil aggregates to wetting. No increase in total C within the soil aggregates was found, however, there were large increases in soil aggregate stability. The rate of incorporation of the C from the added residues differed between plant materials.

**Introduction.** Grain production is of major agricultural importance in Australia, where much of the arable land has been developed from forest or natural grassland. Agricultural development of native lands has led to marked decline in soil organic matter (SOM) throughout the world. This contributes both to global warming via CO<sub>2</sub> evolution from the soil as the SOM mineralizes, and to decline in physical and chemical fertility.

A survey carried out by the Soil Conservation Service of New South Wales in 1987–88 showed that 18% of the state suffered from soil structural decline [1]. Soil structural decline and aggregate breakdown can result in surface sealing, hardsetting, compaction, reduced water infiltration, and increased surface runoff and erosion. Plant growth can be adversely affected. The most obvious effect is on root growth. If the soil is compacted, with few pores through which to pass, root growth can be severely reduced. The development of surface seals through aggregate breakdown can pose considerable mechanical impedance to seedling emergence. Improvement in soil structure can result in increased yields through improved plant growth, better soil-water relations, higher infiltration, and reduced run-off and erosion risk. This has the potential to reduce the possibility of pesticide and herbicide residues, and of soil and nutrients, leaving the farm and entering waterways, and thus lessening the environmental impact of agriculture.

**Materials and methods.** Plant material, which was grown in an atmosphere enriched in carbon isotopes (<sup>13</sup>C/<sup>14</sup>C) and which received <sup>15</sup>N-enriched fertilizer, was incorporated into an Aquic haplustalf soil at the rate of 5 t/ha. Plant residues with a range of breakdown rates [flemingia (*Flemingia macrophylla*), medic (*Medicago trunculata*), and rice (*Oryza sativa*) straw], along with a no-residue-return control, were used. Prior to the commencement of the study, the soil was incubated at field capacity for 20 days at 25°C day (18 h) and 15°C night (6 h). The soil was then mixed and sub-sampled into vials and incubated at a moisture content of 75% of field capacity at temperatures resembling tropical [30°C day/20°C night (12 h)] or temperate [20°C day/10°C night (12 h)] conditions for up to 200 days. Sample vials were removed from the incubation chambers at 80 and 200 days, and the soil air-dried. At each sampling time and at the commencement of the study, total C (C<sub>T</sub>) and N and <sup>13</sup>C and <sup>15</sup>N were determined on an automated carbon and nitrogen analyser/mass spectrometer. A wet-sieving technique was used to determine aggregate stability (expressed as mean weight diameter, MWD). The soil was gently crushed by rolling to pass through a 4-mm sieve before determination of

aggregate stability. Prior to all C measurements and determination of aggregate stability, all visible plant material not within soil aggregates was removed. Preliminary investigations were conducted using a secondary ion mass spectrometer (SIMS) to locate the  $^{13}\text{C}$ -labelled organic matter within the soil aggregates. In addition to the SIMS, an auto-radiography technique and an electron microprobe are being used to determine the position of the  $^{14}\text{C}$  labelled organic matter within the same soil aggregates.

**Results and discussion.** Neither temperature nor residue had any significant effect on soil aggregate  $C_T$  over the 200-day period. However, between 20 and 200 days, the amount of soil aggregate  $C_T$  derived from the added residues increased by 18% with flémíngia and 9% with rice, and decreased by 23% with medic.

Mean weight diameter increased by 73%, 49%, and 27%, respectively, for the medic, rice, and flémíngia treatments, and there was a decrease of 12% in the control, when compared to the MWD of the soil prior to the first incubation period. Mean weight diameter (averaged over all residues and the control) increased by 42% at the higher temperature compared to an increase of 27% at the lower temperature.

Preliminary results from the SIMS scans showed the presence of  $^{13}\text{C}$  within the soil aggregates in the medic and rice treatments. No  $^{13}\text{C}$  was detected in the flémíngia treatment

The decline in the amount of  $C_T$  derived from the medic residue relates to the fast breakdown rate of this plant material [2], which most likely resulted in the release of more labile C compounds, which improved the stability of soil aggregates [3] by providing important binding agents. Following wet sieving of the flémíngia treatment, a large amount of leaf material was visible on the top sieve, compared to the medic and rice treatments, even though all visible material had been removed prior to wet sieving. This indicated that there was a considerable amount of undecomposed leaf material in the aggregates, which became apparent when they slaked during sieving. This undecomposed material would not be involved in the binding of the aggregates against the forces of the water. However, over a longer time, this may decompose and become effective in stabilizing the soil aggregates.

The correct management and incorporation of plant residues can improve aggregate stability and assist in reducing the decline in soil structure. Residues with fast-breakdown rates provide short-term responses in stabilizing soil aggregates, but, over the longer term, residues with slower breakdown rates may be necessary to provide continued improvement in soil structure. To develop sustainable agricultural systems and to reduce decline in soil structure, it is necessary to incorporate plant residues with breakdown rates that suit the environment and provide both short- and long-term stabilization of aggregates.

## ACKNOWLEDGEMENTS

This work was funded by the Grains Research and Development Corporation (GRDC) in Australia and the Australian Institute of Nuclear Science and Engineering (AINSE).

## REFERENCES

- [1] GRETTON, P., SALMA, U., Land Degradation and the Australian Agricultural Industry, Staff Information Paper, Industry Commission, Belconnen, ACT (1996).
- [2] LEFROY, R.D.B., et al., An in vitro perfusion method to estimate rates of plant residue breakdown and associated nutrient release, *Aust. J. Ag. Res.* **46** (1995) 1467–1476.
- [3] BLAIR, N., CROCKER, G.J., Crop rotation effects on soil carbon and physical fertility of two Australian soils, *Aust. J. Soil Res.* **38** (2000) 71–84.

## MICROCOSMS FOR EVALUATION OF DEGRADATION OF $^{14}\text{C}$ XENOBIOTICS IN SOIL

C. IN DER WIESCHE, F. ZADRAZIL  
Institute of Plant Nutrition and Soil Science

R. MARTENS  
Institute of Agroecology

Braunschweig, Germany

The estimation of degradation of xenobiotics in soil (e.g. industrial pollutants) is important for further use of the soil [1]. The best evidence that a microbial community has the ability to mineralize an organic compound undoubtedly is the conversion of  $^{14}\text{C}$ -labelled compounds to  $^{14}\text{CO}_2$  [2].

The objective of this study was to investigate the mineralization of polycyclic aromatic hydrocarbons (PAHs) in soil by white-rot fungi that grow from a lignocellulose substrate into the soil, using microcosms that are easy to handle and simulating natural conditions. The soil was artificially contaminated with a  $^{14}\text{C}$ -labelled compound and inoculated with straw colonized by the fungi in tube reactors [3]. These reactors were connected to an aeration train and continually flushed with  $\text{CO}_2$ -free, moistened and sterile air. The liberated  $^{14}\text{CO}_2$  was trapped in a vessel containing of 2 M NaOH solution. The absorption solution was changed at regular intervals and radioactivity of the solution was estimated in a liquid scintillation counter. A scheme of the experimental design is shown in Fig. 1.

Various kinds of bioreactors with different arrangements of straw and soil compartments were used in order to approach optimum conditions for bioremediation of PAH-polluted soil using white-rot fungi in an on-site treatment. In addition, the aeration was modified in different treatments.

It was found that mineralization of  $^{14}\text{C}$ -PAH in soil by white-rot fungi and indigenous microorganisms decreased with increasing distance from the straw-fungus compartment. As a conclusion, fungal substrate and soil should be mixed carefully to ensure short contact distances. The distance between the single substrate agglomerates that are mixed into the soil should not exceed 5 to 8 cm. Furthermore, the results indicate that active aeration of the straw-soil piles significantly increases PAH-mineralization by white-rot fungi and by indigenous soil microorganisms.

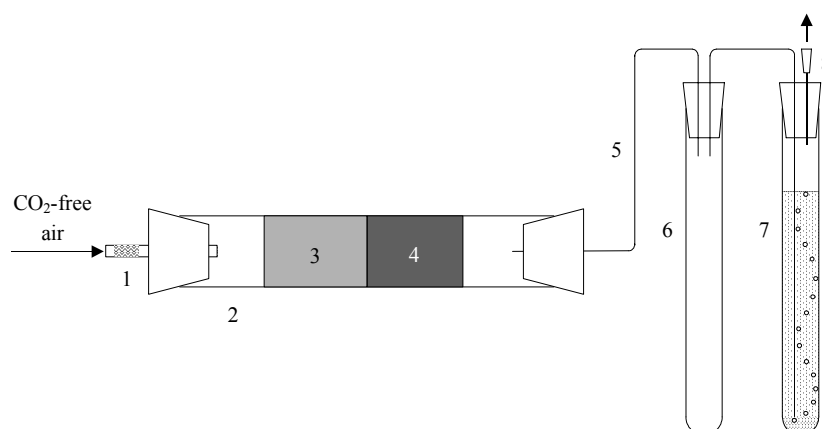


FIG. 1. Scheme of the experimental design. 1: sterilizing filter, 2: tube reactor, 3: chopped wheat straw inoculated with a fungus, 4: soil contaminated with  $^{14}\text{C}$ -labelled compounds, 5: capillary tubing, 6: test tube for prevention of return flow, 7: test tube with 25 mL 2 M NaOH ( $\text{CO}_2$  trap), 8: syringe needle as outlet.

## REFERENCES

- [1] WOOD, P.A., Remediation methods for contaminated soils, *Issues Environ. Sci. Technol.* **7** (1997) 47–71.
- [2] HEAD, I.M., Bioremediation: towards a credible technology, *Microbiology* **144** (1998) 599–608.
- [3] MARTENS, R., ZADRAZIL, F., Screening of white-rot fungi for their ability to mineralize polycyclic aromatic hydrocarbons in soil, *Folia Microbiol.* **43** (1998) 97–103.

## THE USE OF CARBON-ISOTOPE DISCRIMINATION TO SCREEN WHEAT CULTIVARS FOR TOLERANCE OF SALINITY AND DROUGHT

R. SHAHEEN

Institute of Mineralogy and Petrography,  
Lausanne, Switzerland

R.C. HOOD

FAO/IAEA Agriculture and Biotechnology Laboratory,  
Seibersdorf, Austria

Stable carbon isotope determinations provide time-integrated measures of plant physiological activities and plant interactions with the environment. In these experiments the effects of soil salinity on carbon isotope discrimination were studied.

The classical method of selection of wheat cultivars based on yield performance under saline conditions has been largely unsuccessful. Also, physiological traits such as dry matter (DM), water use efficiency (WUE), and harvest index (HI) have been used as alternatives to screening for yield. Carbon-13 discrimination ( $\Delta$ ) is an integrated measure of the response of photosynthetic gas exchange to environmental variables such as water availability, light, humidity, and salinity [1], and has been shown to be a useful tool in the selection of cultivars for drought tolerance.

Despite similarities between the effects of water and salt stresses on plant growth, few attempts have been made to quantify the effect of salinity on  $\Delta$ , and its potential as a breeding selection characteristic, aimed at increasing grain yield under saline conditions.

The objectives of this experiment were to study the effect of soil salinity on  $\Delta$  in salt- and drought-tolerant wheat cultivars under well watered and water-limiting conditions, and to evaluate the relationship between  $\Delta$ , DM, WUE, and HI under the two levels of moisture.

The experiment was set up in a factorial design, with two watering regimes [35% and 75% plant available water (PAW)] and four salinity levels (0, 8, 12, 16 dS/m), with two salt-tolerant (Karchia CWI10990, Shorawaki BW20313) and two drought-tolerant (Pastor CM85836, Baviacora BW18103) cultivars of wheat. The experiments were conducted in the glasshouse at the FAO/IAEA Seibersdorf Laboratories. Micro-porous cup samplers were installed in each pot and soil salinity was periodically measured using a conductivity meter. Plants were harvested at maturity, dried at 70°C, and DM, WUE (dry matter/total water applied), and HI (grain yield/above-ground dry matter) were determined.

Leaf disks were collected from flag leaves using a hole puncher, and dried at 70°C. Carbon-isotope ratios were measured using mass spectrometry (Micromass, Optima). Carbon-isotope discrimination ( $\Delta$ ) was calculated according to Ref. [1], assuming an isotope composition of ambient air of -8.0‰. Statistical analyses were carried out using ANOVA.

Carbon-isotope discrimination ( $\Delta$ ) varied significantly ( $P < 0.001$ ) among cultivars under both watering regimes. Soil salinity produced a linear and significant ( $P < 0.001$ ) decrease in  $\Delta$  in all of the cultivars under wet and dry conditions (Figs. 1 and 2).

Under water-sufficient conditions,  $\Delta$  had a range of 2.14‰, and the range was narrower, 1.76‰, under water-stressed conditions. A cultivar  $\times$  salinity interaction was significant for  $\Delta$ . An interaction between cultivar  $\times$  salinity observed for HI was also significant ( $P < 0.05$ ), indicating strong genotypic differences in response to salt treatment.

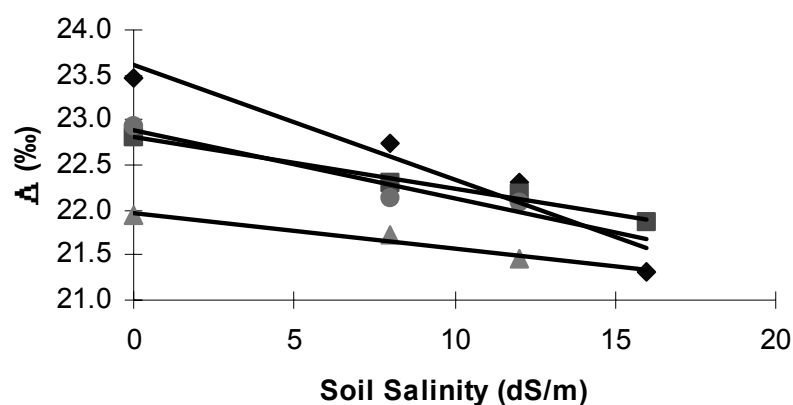


FIG. 1.  $\Delta$  values of four wheat cultivars in moisture-sufficient conditions (see legend in Fig. 2).

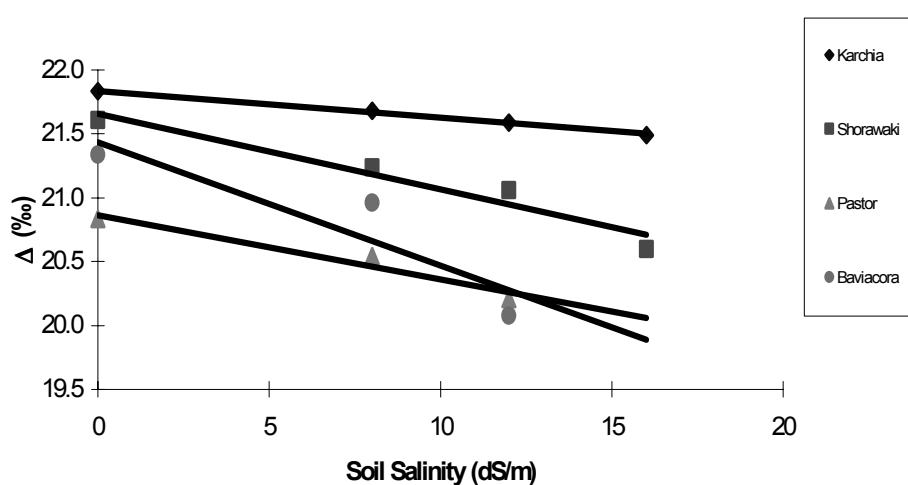


Fig 2.  $\Delta$  values of four wheat cultivars in moisture-limiting conditions.

A positive phenotypic correlation was found between  $\Delta$  and HI for the salt-tolerant cultivars, suggesting that high  $\Delta$  may be used to indirectly select for higher grain yield in wheat under water-sufficient conditions. Variation in  $\Delta$  observed for the cultivars under salt stress was probably due to variation in photosynthetic capacity rather than stomatal conductance alone, as indicated by a negative relationship between  $\Delta$  and biomass under moisture-deficient conditions.

The phenotypic correlation between  $\Delta$ , DM, and WUE varied according to the pattern of stress. These correlations were negative under dry conditions and became positive under moist conditions.

## REFERENCE

- [1] FARQUHAR, G.D., RICHARDS, R.A., Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes, Aust. J. Plant Physiol. **11** (1984) 539–552.



## **LABELLING OF SEWAGE SLUDGE WITH $^{13}\text{C}$ AND $^{15}\text{N}$ ISOTOPES**

H. KIRCHMANN, Y. COHEN

Swedish University of Agricultural Sciences,  
Uppsala, Sweden

Treatment of sewage water varies with the type and level of technology applied. As a result, sewage sludges vary in composition. In Western Europe, a combination of mechanical, biological, and chemical treatments is commonly applied. The biological treatment of sewage water—the activated sludge process—results in removal of carbon and nitrogen through immobilization in microbes. With strong aeration of the wastewater, energy-rich substrates and nutrients are assimilated by aerobic microbes and a large microbial biomass results. The biomass consists mainly of living microbial cells and components of dying and dead cells, but also of colloidal particles and metal ions bound to the surfaces of the microbes. The organic matter produced during aeration—the biological sludge—is removed by settlement. The biological treatment of wastewater was the starting point for the labelling procedure of sewage sludge.

Labelling of waste products with stable tracer isotopes can be done in two ways: (i) labelling of the original material from which wastes are generated, e.g. by labelling of the diet fed to animals [1, 2]; and (ii) labelling during the biological turnover through addition of nitrogen or carbon compounds to wastes [3]. In this study, tracers were added to wastewater during biological treatment.

Wastewater was sampled from the activated sludge process at the Kungsängenverk sewage plant in Uppsala, and transferred to the laboratory. The samples, consisting both of water and of suspended sludge had high concentrations of carbon, nitrogen, and phosphorus. Quantities of about 10 L of wastewater were used for the labelling experiments. By adding  $^{15}\text{N}$ -enriched ammonium sulphate, the ammonium part of the wastewater was labelled. The rate used was 1.25 g ammonium sulphate (99%  $^{15}\text{N}$ )  $\text{L}^{-1}$ . Three treatments were tested: (i) addition of ammonium sulphate only; (ii) addition of ammonium sulphate and glucose; (iii) addition of ammonium sulphate and split applications of glucose. Glucose, an additional energy source for the microflora in the wastewater, was added at a rate of 30 g  $\text{L}^{-1}$  in total. With the split applications, batches of 10 g glucose  $\text{L}^{-1}$  were added three times. The glucose-enriched wastewater was aerated at the high flow rate of about 1  $\text{L min}^{-1}$  at a constant temperature of 20°C for 7 days to maintain aerobic conditions and mimic an activation process. Thereafter, the suspension was allowed to settle and the water phase was removed. The sludge was dried at 30°C and analysed.

Significant differences between treatments with and without glucose were observed. Glucose addition, whether through split or full application, resulted in the formation of a different microflora as compared to the ammonium sulphate-only treatment. Although the microflora was not investigated as such, the very different odour, colour and the intensive foaming of the wastewater upon glucose addition showed that C labelling through glucose addition would not be representative. The biological sludge recovered at the end of the experimental period had a much higher C:N ratio, 25, the C content was significantly higher, 58%, and the total amount sludge produced was 20% less than in the ammonium sulphate treatment (Table I).

TABLE I. MEAN ANALYSIS VALUES OF LABELLED SEWAGE SLUDGE PRODUCED THROUGH ADDITION OF  $^{15}\text{N}$  LABELLED AMMONIUM SULPHATE ADDED TO BIOLOGICAL SLUDGE BEFORE AERATION.

Addition	Organic C (%)	Total N (%)	C:N	$^{15}\text{N}$ (atom %)	$^{15}\text{N}$ recov. (%)
Ammonium sulphate	20	3.6	5.4	3.630	2.0
Ammonium sulphate +glucose	58	2.4	25	5.401	1.6

It was concluded that glucose addition is not a suitable way to produce  $^{13}\text{C}$  labelled sewage sludge. At this stage, no suggestion for a more suitable C compound can be offered.

Samples of sewage sludge with ammonium sulphate addition had a C:N ratio of 5.4, an N content of 3.7% and a C content of 20%, which are similar to values obtained in unlabelled sewage sludge. Although the analyses are promising, the recovery of added  $^{15}\text{N}$  was low, on average about 2% (Table I). This means that the intensive turnover during aeration caused large losses of N probably both through nitrification/denitrification and through ammonia volatilization.

### ACKNOWLEDGEMENT

The authors wish to express their gratitude to the International Atomic Energy Agency for funding this study as a technical project (Contract No. 10561).

### REFERENCES

- [1] KIRCHMANN, H., Losses, plant uptake and utilisation of manure nitrogen during a production cycle, *Acta Agric. Scand. Supplementum* **24** (1985) 1–77.
- [2] SÖRENSEN, P., et al., Labelling of animal manure nitrogen with  $^{15}\text{N}$ , *Plant Soil* **162** (1994) 31–37.
- [3] GUTSER, R., DOSCH, P., “Cattle slurry— $^{15}\text{N}$  turnover in a long-term lysimeter trial”, *Fertilizers and Environment* (RODRIGUEZ-BURRUECO, C., Ed.), *Dev. Plant Soil Sci.* **66** (1996) 345–350.

## NITROGEN TRANSFER FROM LEGUMES TO NON-LEGUMES

G. HARDARSON, M. AIGNER

FAO/IAEA Agriculture and Biotechnology Laboratory,  
Seibersdorf, Austria

**Introduction.** Several isotope techniques have been used to measure N transfer from legumes to non-legumes including  $^{15}\text{N}$  dilution,  $^{15}\text{N}_2$  labelling, split-root  $^{15}\text{N}$  labelling, and leaf or stem  $^{15}\text{N}$  feeding. Most of these methods have shown very little or no direct transfer of N from legumes to non-legumes when grown in mixed cropping systems. However, some studies have been able to quantify N rhizodeposition by legumes and N transfer when the legume root system is decomposing, e.g. during or after cutting or stress. In the present greenhouse study, we investigated the time course of N transfer from soybean [*Glycine max* (L.) Merr.] and common bean (*Phaseolus vulgaris* L.) to associated wheat (*Triticum aestivum*) using the stem  $^{15}\text{N}$ -feeding technique. The objective was to determine if N transfer occurred during any of the various growth stages of the legumes.

**Material and methods.** A greenhouse experiment was conducted at the Seibersdorf Laboratory with pots, each containing a 4-kg Seibersdorf-soil:quartz-sand mixture (1:1). Each pot was sown with one inoculated soybean (cv. Clay) or common bean (cv. Red Kidney) plant, and with five adjacent wheat (cv. Capo) plants around the legume as shown in Fig. 1. The stem  $^{15}\text{N}$ -labeling technique of McNeil [1] was used to label the legumes. Two mL of 0.075 M urea (~20%  $^{15}\text{N}$  atom excess) solution were taken up by each legume plant, as shown in Fig. 2. The legumes were labelled at 14, 19, 26 or 32 days after planting (DAP); only the data from the 14-DAP labelling are shown. Single wheat plant were harvested at weekly intervals and dry matter yield, total N, and %  $^{15}\text{N}$  atom excess were determined. After 6 weeks, the legumes were cut and the  $^{15}\text{N}$ -labelled roots left in the soil. The wheat plants were allowed to regrow to investigate N uptake from decomposing legume roots.

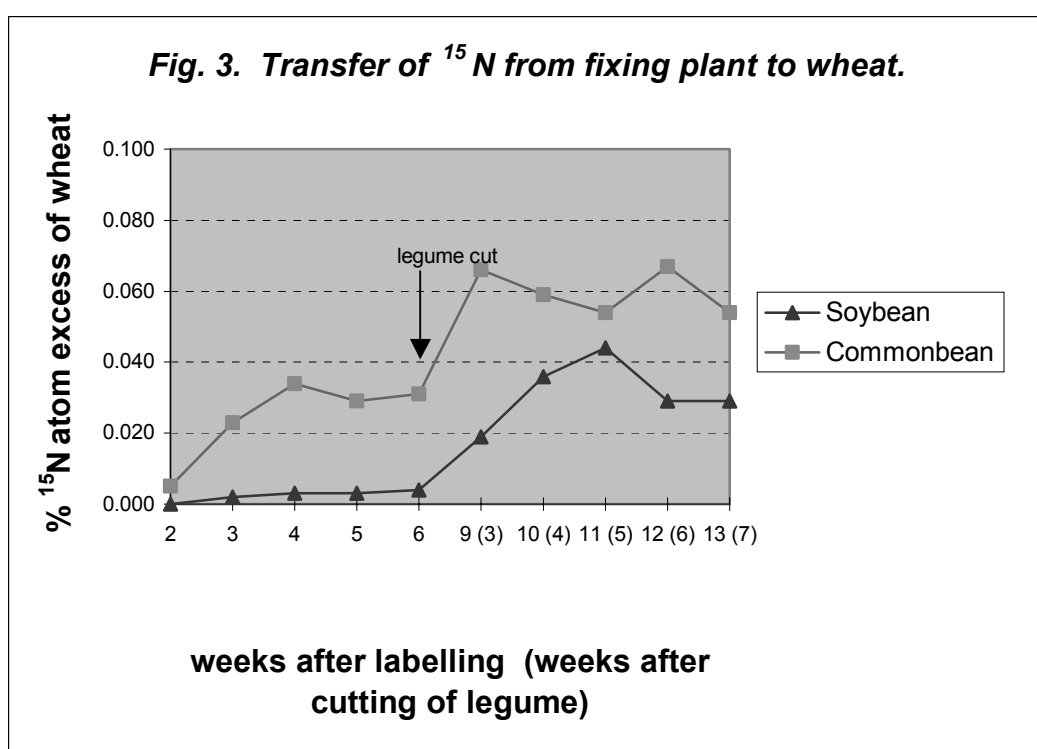
**Results.** The  $^{15}\text{N}$  stem-feeding technique was successful in labelling the leguminous plants (soybean: 0.5% and common bean: 0.9%  $^{15}\text{N}$  atom excess). Hardly any transfer of  $^{15}\text{N}$  from soybean to the adjacent wheat plants was observed (Fig. 3, weeks 2 to 6) whereas a significant amount of  $^{15}\text{N}$  was transferred from common bean plants to wheat throughout the growing period. Also, significant amounts of  $^{15}\text{N}$  were taken up by the wheat plants from the decomposing roots of soybean and common bean (Fig. 3, weeks 9 to 13).



FIG. 1. Experimental set-up.



FIG. 2.  $^{15}\text{N}$  stem labelling.



The growth stage of the legumes had little influence on the rate of  $^{15}\text{N}$  transfer from the legume to the non-legume. However the adjacent wheat benefited more from N derived from common bean as compared to soybean from which very little N was transferred during growth. Percent N in wheat derived from common bean was approximately 3% during the growing period of the legume, compared to approximately 7% after the legume had been cut. Further studies are warranted to compare the ability of other legumes and cultivars to supply N to adjacent plants.

## REFERENCE

- [1] McNEIL, A., Enriched stable isotope techniques to study soil organic matter accumulation and decomposition in agricultural systems”, Application of Stable Isotope Techniques to Study Plant Physiology, Plant Water Uptake and Nutrient Cycling in Terrestrial Ecosystems (Unkovich, M., Ed.), Centre for Legumes in Mediterranean Agriculture, Perth (1999) 105–121.

## CHANGE OF $\delta^{15}\text{N}$ NATURAL ABUNDANCE ( $\delta^{15}\text{N}$ ) IN A FOREST SOIL RECEIVING ELEVATED N DEPOSITION

H. VERVAET, P. BOECKX, O. VAN CLEEMPUT, G. HOFMAN  
Ghent University  
Gent, Belgium

**Introduction.** Natural abundance of  $\delta^{15}\text{N}$  ( $\delta^{15}\text{N}$ ) has been used to interpret N mineralization in forest ecosystems. Forest litter typically has depleted  $\delta^{15}\text{N}$  values ranging from  $-8$  to  $0\text{‰}$  [1, and references within] and  $\delta^{15}\text{N}$  values of organic N in forest soil profiles become more enriched with depth [2–4].

This study investigated (1) the change of  $\delta^{15}\text{N}$  ( $\text{‰}$ ) and total N (TN,  $\text{‰}$ ) with depth, and (2) the relation between the change of  $\delta^{15}\text{N}$  ( $\Delta\delta^{15}\text{N}$ ) within the 0 to 10, 10 to 20 and 20 to 30 cm intervals of the mineral layer and the N mineralization rates in these layers.

**Methodology.** Samples were taken at three locations in a mixed deciduous forest, receiving an average deposition of  $36 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ : one location was at the forest edge (E) and two were deeper in the forest (F1, F2). The litter layer (L), the fermentation plus humus (F+H) layer, and the mineral layer (0–30 cm) were sampled at intervals of 2 cm. In these intervals  $\delta^{15}\text{N}$  ( $\text{‰}$ ) and TN ( $\text{‰}$ ) were determined in duplicate with a EUROPA PDZ ANCA-SL elemental analyser connected with a continuous-flow isotope ratio mass spectrometer (model 20-20 EUROPA-PDZ).

Mineralization rates ( $\text{mg N m}^{-2} \text{ d}^{-1}$ ) for the mineral layers (0–10, 10–20 and 20–30 cm) were determined by laboratory incubation experiments at  $15^\circ\text{C}$  and 50% WFPS. The changes in  $\delta^{15}\text{N}$  values in 10-cm increments were calculated ( $\Delta\delta^{15}\text{N}$ ).

**Results.** Figure 1 shows that the  $\delta^{15}\text{N}$  values increased with depth. In the organic layers (L and F+H),  $\delta^{15}\text{N}$  values varied between  $-11.9$  and  $-6.5\text{‰}$ . For the mineral layer, the  $\delta^{15}\text{N}$  values ranged from  $-7.4$  to  $+2.0\text{‰}$ . The  $\delta^{15}\text{N}$  values became constant at  $-10$  cm for locations E and F2 and at  $-25$  cm for location F1. At the same depths, mineralization rates decreased sharply.

Enrichment factors ( $\epsilon$ ), calculated according to Mariotti et al. [5], varied from  $-1.9$  to  $-5.4\text{‰}$  (Fig. 2). These factors indicate faster mineralization of isotopically light organic matter and enrichment of residual soil organic matter in  $^{15}\text{N}$ .

A linear regression was fitted through the mineralization rates of the different mineral layers from the three locations (E, F1 & F2) and the  $\Delta\delta^{15}\text{N}$  values of the same layers (Fig. 3). The regression was significant ( $R^2 = 0.79$ ,  $P < 0.001$ ) when fitted through the origin, confirming that  $\delta^{15}\text{N}$  values became constant when mineralization stopped.

Mineralization rates of the 0 to 30 cm mineral layers versus the enrichment factors also gave a strong linear relation, but further research is needed because little data were available.

**Conclusions.** The  $\delta^{15}\text{N}$  values increased with depth, ranging from  $-11.9$  to  $-6.5\text{‰}$  for the organic layers and from  $-7.4$  to  $+2.0\text{‰}$  for the mineral layer.

Enrichment factors ranged from  $-1.9$  to  $-5.4\text{‰}$ , indicating faster mineralization of isotopically light organic matter and enrichment of residual soil organic matter in  $^{15}\text{N}$ .

Very significant linear relations were found between N mineralization rates and  $\Delta\delta^{15}\text{N}$  on one hand, and enrichment factors on the other hand. Further research will be developed to investigate whether changes in  $\delta^{15}\text{N}$  or  $\epsilon$  values can be used to predict N-mineralization behaviour of forest soils.

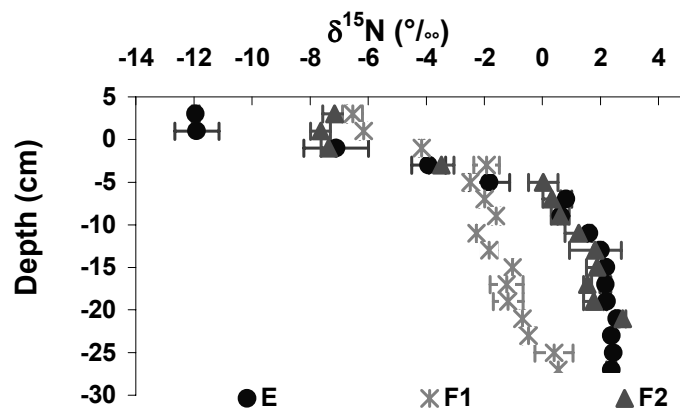


FIG. 1. Changes in  $\delta^{15}\text{N}$  (‰) with depth.

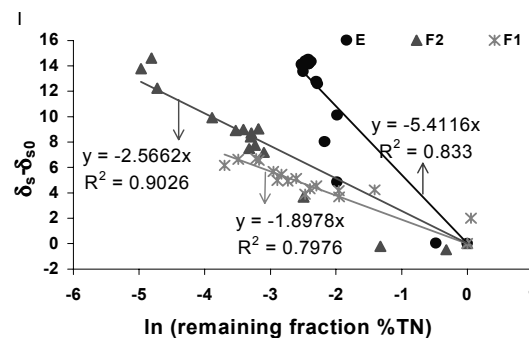


FIG. 2. Enrichment factors for the three locations.

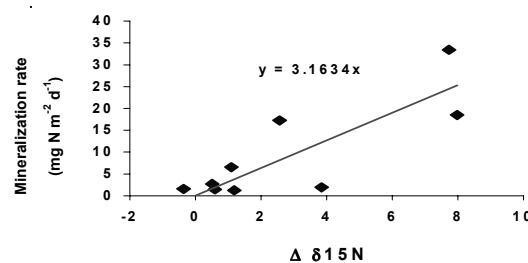


FIG. 3. Relation between mineralization rates and  $\Delta\delta^{15}\text{N}$  values.

## REFERENCES

- [1] NADELHOFFER, K.J., FRY, B., Controls on natural Nitrogen-15 and Carbon-13 abundances in forest soil organic matter, *Soil Sci. Soc. Am. J.* **52** (1988) 1633–1640.
- [2] KOOPMANS, C.J., et al., Natural  $^{15}\text{N}$  abundance in two nitrogen saturated forest ecosystems, *Oecologia* **111** (1997) 470–480.
- [3] BUZEK, F., et al., The behaviour of nitrogen isotopes in acidified forest soil in the Czech Republic, *Water Air Soil Poll.* **105** (1998) 155–164.
- [4] EMMETT, B.A., et al., Natural abundance of  $^{15}\text{N}$  in forests across a nitrogen deposition gradient, *Forest Ecol. Management* **101** (1998) 9–18.
- [5] MARIOTTI, A., et al., Experimental determination of nitrogen kinetic isotope fractionation: some principles; illustration for the denitrification and nitrification processes, *Plant Soil* **62** (1981) 413–430.



## CLOSING STATEMENT AND SYNTHESIS

**P.M. Chalk**

Head

Soil and Water Management & Crop Nutrition Section  
Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture  
International Atomic Energy Agency  
Vienna

One hundred and seventeen participants representing forty-three countries and five organizations have taken part in this symposium. Recent advances in the development and application of nuclear-based techniques have been highlighted and trends and future issues identified. The presentations have once again emphasized the role that nuclear-based techniques can play in gaining unique information that adds considerable value to studies in nutrient and water dynamics. Attention has also been drawn to the fact that major advances in knowledge often follow closely behind advances in methodology. We have had many good examples of such developments in this symposium. It is also clear from the contributions that there is great breadth in the range of nuclear techniques at our disposal, particular in the use of isotopes as tracers in estimating nutrient fluxes. However, it is also clear that there are increasing restrictions on the field use of radionuclides and the neutron moisture probe. Where possible, stable isotopes are replacing radionuclides as tracers to follow nutrient dynamics, e.g.  $^{13}\text{C}$  vs.  $^{14}\text{C}$  and  $^{34}\text{S}$  vs.  $^{35}\text{S}$ . The increased use of  $^{13}\text{C}$  in the last decade has indeed been phenomenal, while  $^{15}\text{N}$  continues to play an essential role in nitrogen studies. Advances in instrumentation for stable isotope analysis during the last decade have also been remarkable. We have seen major developments in sample automation and increased sensitivity. New analytical techniques have been developed to take advantage of the new instrumentation. However, it should be pointed out that isotope ratio analysis is neither simple nor cheap, and it is beyond the reach of many scientists in developing countries. The Agency has an important role to play here in the provision of analytical services, quality assurance, the provision of equipment and fellowship training.

A synthesis of the sessions of the symposium from notes supplied to me by the respective chairpersons and from my own perspective follows.

### **Session 1: Evaluation and management of natural and manufactured nutrient sources**

Innovations in the use of radioisotopes and natural isotopic abundance in nutrient cycling and catchment scale studies were highlighted in the keynote paper, and the need to consider the interaction between carbon and other nutrients was stressed. Papers on the use of the indirect  $^{15}\text{N}$ -dilution method to estimate N release from green manures, crop residues, and sewage sludge were presented. Developments in the use  $^{32}\text{P}$ -exchange kinetics to evaluate nutrient sources and the effectiveness of management practices were outlined. In-situ labelling with  $^{32}\text{P}$  continues to play an important role in studying root extension and P-uptake patterns by plants. Information is still emerging on aspects of the soil N cycle, as shown by papers in both Sessions 1 and 2. Dissimilatory reduction of nitrate to ammonium in reduced environments can be much higher than previously believed. Similarly, direct assimilation of organic N into microbial tissues during decomposition of crop residues is a major pathway. This finding suggests that the role of autotrophic vs. heterotrophic nitrification should be further investigated.

### **Session 2: Soil organic matter dynamics and nutrient cycling**

The application of isotopic techniques in this field is very large, as outlined by the keynote speaker who stressed the importance of the interaction of C and N, and how  $^{15}\text{N}$  and  $^{13}\text{C}$  are being used to study this interaction. The speaker also highlighted future challenges including a better understanding of the effect of organic matter on soil structure and the processes of chemical and physical stabilization of organic carbon. Several papers showed the importance of measuring gross rates of



mineralization and immobilization to understand measured net effects, and it is interesting to note the recent interest in this methodology which was developed in 1954 and virtually ignored for several decades. Other innovative applications of  $^{15}\text{N}$  were the use of natural abundance to assess denitrification in buffer strips, and foliar labelling to assess below-ground plant-derived N. This latter work shows clearly that we have underestimated this input by non-isotopic methods and that we need to revise our estimates of the role of legumes in the N economies of cropping systems. The use of  $^{13}\text{C}$  natural abundance to estimate carbon turnover rates is a powerful tool that can be applied with some success in mixed C3/C4 rotations and also to trace the fate of residue carbon into various biological, physically and chemically defined pools of soil organic matter.

### **Session 3: Soil water management and conservation**

This session covered the measurement of moisture in soils, water use efficiency, water balance and fertilizer N efficiency in relation to water use and fertigation. The following conclusions were drawn:

- The utility of the soil moisture neutron probe was outlined in the keynote, with a timely reminder of the importance of correct calibration. Comparative assessment with non-nuclear methodologies is essential and guidelines for their respective applications are needed. The Agency is supporting work on both aspects.
- The neutron probe, combined with other measurements, is an essential tool in estimating soil water balance in natural and agricultural ecosystems and for irrigation scheduling.
- Major savings in both water and N fertilizer can be achieved through drip irrigation systems. However, the acidification hazard for perennial crops on poorly buffered soils and cost-benefit analysis both need to be determined.

### **Session 4: Plant tolerance to environmental stress**

Topics covered in this session were:

- A description of the IAEA Interregional Model TC Project on the use of saline groundwater to irrigate wastelands for production of crops, fodder and fuel wood.
- The application of the  $\delta^{13}\text{C}$  technique to provide essential information on water use by desert plant communities for sustainable ecosystem management.
- The sensitivity of different plant C pools to changes in the  $\delta^{13}\text{C}$  signature under drought stress.

The  $\delta^{13}\text{C}$  technique is likely to play an increasingly important role in screening crop genotypes for tolerance to environmental stress, particularly drought and salinity.

### **Session 5: Environmental and pollution studies**

Highlighted were the latest analytical techniques in the area of neutron activation analysis, radiometric analyses, trace gas analyses, and tracer techniques and their application to quantify the fate of inorganic and organic pollutants under a range of ecosystems. With the adoption of new techniques with lower detection limits, it is possible to partition pollutants between solid and solution phases in soils, and thus improve the prediction of the transfer of pollutants through the food chain.

Future issues and trends in environmental pollution studies are likely to include:

- The use of isotopes with lysimeters or other models to develop tools that can accurately predict the movement of pollutants through the ecosystem, uptake by plants and or degradation in the environment.
- The use of multiple isotopic analyses (e.g.  $^{15}\text{N}/^{18}\text{O}$ ) to assess pollution in catchment-scale studies.
- The integration of the improved capacity to partition pollutants in ecosystem components with risk assessment studies.

## **Session 6: Assessment of soil erosion and sedimentation**

Recent developments in the application of the  $^{137}\text{Cs}$  technique were highlighted and included:

- Refined procedures to establish reference inventories.
- Development, testing and validation of calibration models for converting  $^{137}\text{Cs}$  measurements into soil-redistribution rates.
- Application of the methodology to Chernobyl-affected areas.
- Extension of the technique to other environmental radionuclides such as unsupported  $^{210}\text{Pb}$  and  $^7\text{Be}$ .
- The successful implementation of two IAEA Co-ordinated Research Projects aimed at standardizing procedures for using  $^{137}\text{Cs}$  in soil-erosion and sedimentation studies, paving the way to expand the scope of the technique to other issues such as validation of erosion and sedimentation distributed models, establishment of soil erosion-crop productivity relationships and the assessment of soil-conservation strategies.

## **Session 7: Recent advances in isotope analytical methodologies and related instrumentation**

The keynote speakers conveyed the dynamic sense of research and development in instrumentation, outlining exciting innovations and future trends in the analysis of radioactive and stable isotopes. Many of these new techniques simplify sample analyses and increase sample throughput. These new developments are well attuned to the specific needs of scientists working in the agricultural and environmental sciences.

### **Poster session:**

Thirty-eight of the scheduled forty-four posters, covering a range of topics, were on display.

- Nineteen posters were concerned with aspects of nitrogen, twelve of which dealt with N cycling and plant nutrition.
- Several posters in each of the following areas were displayed: sewage sludge, C, P and water.
- Individual posters on micronutrients, Cs, uranium, and the Diviner 2000<sup>®</sup> capacitance probe were displayed.

The session was well attended by the authors and participants. Lively discussions ensued at many of the posters indicating that the session achieved its objective of information exchange.

## **ACKNOWLEDGEMENTS**

I would like to thank the contributors to all of the sessions, especially the keynote speakers and the chairpersons of each session. K. Morrison, Conference Service Section, provided crucial support in implementing and facilitating the symposium. She was assisted by L. Algad, S. Chung, and A. Glannar. L. Kruzic, Soil and Water Management & Crop Nutrition Section, provided invaluable secretarial assistance to the Scientific Secretary.



## SYMPOSIUM OFFICIALS

### CHAIRPERSONS OF SESSIONS

Opening Session	J.D. DARGIE	FAO/IAEA
Session 1	C. HERA J.C. FARDEAU	Romania France
Session 2	Z.A. RAHMAN E.S. JENSEN	Malaysia Denmark
Session 3	O. VAN CLEEMPUT	Belgium
Session 4	R. BILAL	Pakistan
Session 5	C.J. SMITH	Australia
Session 6	J. ROGASIK	Austria
Session 7	M. GERZABEK	Austria

### SECRETARIAT OF THE SYMPOSIUM

P.M. CHALK	Scientific Secretary (FAO/IAEA)
K. MORRISON	Symposium Organizer (IAEA)



## LIST OF PARTICIPANTS

### AFGHANISTAN

Tutakheil, N. Faculty of Natural Sciences, Kabul University, Kabul  
unvco.kabul@undpafg.org.pk

### ARGENTINA

Lopez, S. Unidad de Actividad, Aplicaciones Tecnologicos y Agropecuarias,  
Comision Nacional de Energia, Atomica Av. del Libertador 8250,  
1429 Buenos Aires  
silopez@cae.cnea.gov.ar

Magnavacca, C.A. Ezeiza Atomic Center, Comisión Nacional de Energia Atómica,  
Pbro. Gonzalez y Aragón 15, 1804 Ezeiza  
fax: 541143798322  
cecimag@cae.cnea.gov.ar

Videla, C.C. Facultad de Ciencias Agrarias, Universidad Nacional de Mar del  
Plata, Ruta 226 km 74.6, C.C. 276, (7620) Balcarce  
fax: 542266439101 and 439140  
cvidela@balcarce.inta.gov.ar

### AUSTRALIA

Blair, G. Agronomy and Soil Science, University of New England,  
Armidale, NSW 2350

Blair, N. Agronomy and Soil Science, University of New England,  
Armidale, NSW 2350

Buss, P. Sentek Pty Ltd., 77 Magill Road, Stepney, SA 5069  
fax: 61883628400  
pbuss@sentek.com.au

Chen, D. Institute of Land and Food Resources, University of Melbourne,  
Victoria 3010  
fax: 6138445037  
d.chen@landfood.unimelb.edu.au

Evans, J. Agricultural Insitute, NSW Agriculture, Private Mail Bag,  
Wagga Wagga, NSW 2650  
fax: 61269381809  
jeffrey.evans@agric.nsw.gov.au

Sanchez-Basilio, P. Agronomy and Soil Science, University of New England,  
Armidale, NSW 2351  
fax: 6126773 3465  
pbasilio@metz.une.edu.au

Smith, C. CSIRO Land and Water, GPO Box 1666, Canberra, ACT 2601  
fax: 61262465965  
cj.smith@cbr.clw.csiro.au



Huber, E.	Institute of Ecology and Conservation Biology, University of Vienna, Althanstrasse 14, A-1090 Vienna ehuber@pflaphy.pph.univie.ac.at
Kaniak, A.	Institute of Ecology and Conservation Biology, University of Vienna, Althanstrasse 14, A-1090 Vienna akaniak@pflaphy.pph.univie.ac.at
Krenn, A.	Austrian Research Centre, A-2444 Seibersdorf fax: 4322547803653 andreas.krenn@arcs.ac.at
Maringer, S.	Institute of Ecology and Conservation Biology, University of Vienna, Althanstrasse 14, A-1090 Vienna smaring@pflaphy.pph.univie.ac.at
Menasse, T.	Institute of Ecology and Conservation Biology, University of Vienna, Althanstrasse 14, A-1091 Vienna menasse@pflaphy.pph.univie.ac.at
Pietsch, G.	Institute of Organic Farming, University of Agriculture, Gregor Mendelstrasse 33, A-1180 Vienna fax: 431476543792 gpietsch@edv1.boku.ac.at
Richter, A.	Institute of Ecology and Conservation Biology, University of Vienna, Althanstrasse 14, A-1090 Vienna fax: 31336776 andreas.Richter@univie.ac.at
Shinonaga, T.	Austrian Research Centre, A-2444 Seibersdorf fax: 4322547803653 taeko.shinonaga@arcs.ac.at
Wanek, W.	Institute of Ecology and Conservation Biology, University of Vienna, Althanstrasse 14, A-1090 Vienna fax: 31336-776 wolfgang.wanek@univie.ac.at
Wania, R.	Institute of Ecology and Conservation Biology, University of Vienna, Althanstrasse 14, A-1090 Vienna fax: 31336-776 rwania@pflaphy.pph.univie.ac.at
Watzka, M.	Institute of Ecology and Conservation Biology, University of Vienna, Althanstrasse 14, A-1090 Vienna fax: 31336-776 margaret@pflaphy.pph.univie.ac.at

## **BANGLADESH**

Ahmed, S.	Bangladesh Institute of Nuclear Agriculture, P.O. Box No. 4, Mymensingh
-----------	----------------------------------------------------------------------------



## **BELARUS**

Tarasiuk, S.                      Research Institute for Soil Science and Agrochemistry,  
Brissa Kazintsa strasse 62, 220108 Minsk  
fax: 375172774480  
tarasiuk@mail.belpak.by

## **BELGIUM**

Boeckx, P.                      Laboratory of Applied Physical Chemistry, University of Ghent,  
Coupure 653, B-9000 Gent  
fax: 3292646230  
pascal.boeckx@rug.ac.be

Dhondt, K.                      Dept. Applied Analytical & Physical Chemistry, University of Ghent,  
Coupure Links 653, B-9000 Gent  
fax: 3192646230  
karel.dhondt@rug.ac.be

van Cleemput, O.O.J.                      Faculty of Agriculture and Applied Biological Sciences,  
University of Ghent, Coupure links 653, B-9000 Gent  
fax: 32 9 2646242  
oswald.vancleemput@rug.ac.be

Vervaet, H. L.                      Faculty of agricultural and Applied Biological Sciences,  
University of Ghent, Coupure 653, B-9000 Gent  
fax: 3292646247  
hilde.vervaet@rug.ac.be

## **BENIN**

Houngandan, P.                      Institut National des Recherches Agricoles du Benin,  
B.P.No. 884, Cotonou  
fax: 229350556  
Phoungandan@yahoo.com

## **BRAZIL**

Alves, B.                      Embrapa Agrobiologia, Km 47 Ant. Rod. Rio-São Paulo,  
23.851-970 Seropedica, Rio de Janeiro  
fax: 55216821230  
bob@cnpab.embrapa.br

Cassaró, F.                      CENA/USP, Av. Centenario, 303 - CP 96, CEP13400-970, São Paulo  
fax: 5519 4294610  
fcassaró@cena.usp.br

Muraoka, T.                      CENA/USP, Avenida Centenário, 30313400-970,  
Piracicaba-São Paulo  
fax: 55 19 4294720  
Muraoka@cena.usp.br

Piasentin, R.M.                      Radiochemistry Division, IPEN/CNEN-SP,  
Caixa Postal 11049-Pinheiros, 05508-900 São Paulo  
fax: 55118169188  
rmpiasen@curiango.ipen.br

## **BULGARIA**

Kamenova-Totzeva, R.M. National Centre of Radiobiology and Radiation Protection (NCRR),  
132 Kl. Ochridsky Blvd., 1756 Sofia  
fax: 3592621059  
ncrrp@medicalnet-bg.org

Nitcheva, O. Institute of Water Problems, Bulgarian Academy of Sciences,  
1 Academy G. Bouchev Str., 1113 Sofia  
fax: 3592 722 577  
nitcheva@bgcict.acad.bg

## **BURKINA FASO**

Bado, B.V. Institut de l'Environnement et de Recherche Agricole (INERA),  
03 BR 7192 Onagadougou 03  
fax: 970159  
vbado@fasonet.bf

## **CHILE**

Rouanet, J.L. INIA, Casilla 58-D, Temuco  
fax: 56 2 3646279  
ipino@cchen.cl

## **CHINA**

Cai, G. Institute of Soil Science, Chinese Academy of Sciences,  
P.O. Box 821, Nanjing  
fax: 08625 3353590  
gxcai@issas.ac.cn

Wang, Jia Yu Solid and Fertilizers Institute, Zhejiang Academy of Agricultural  
Sciences, 198 Shi Qiao Road,  
Hangzhou 310021, Zhejiang Province  
fax: 865716400481  
wjy@mail.hz.zj.cn

Wei, Dongpu Institute for Application of Atomic Energy, Chinese Academy of  
Agricultural Sciences, P.O. Box 5109,  
Beijing 100094  
fax: 86010 62896314  
814wby@ihw.com.cn

Xie, Liqing Institute of Low Energy Nuclear Physics, Beijing Normal University,  
Beijing 100875  
LQXI@263.net

## **COTE D'IVOIRE**

Koffi, N. CNRA, 01 B.P. 1740, Abidjan 01  
fax: 22532 76 08 35  
cnra@aviso.ci or cnra@africaonline.co.ci

## **CUBA**

Dueñas, G. Soil Institute, Autopista Costa Costa y Ant., Carretera de Vento,  
CP 10800, Capdevila, Boyeros, Ciudad de la Habana  
larenee@ceniai.inf.cu

Garcia, A. Soil Institute, Autopista Costa Costa y Ant., Carretera de Vento,  
CP 10800, Capdevila, Boyeros, Ciudad de la Habana  
larenee@ceniai.inf.cu

## **CYPRUS**

Papadopoulos, I. Agriculture Research Institute, P.O. Box 2016, 1516 Nicosia,  
fax: 3572 316770  
papado@arinet.ari.gov.cy

## **DENMARK**

Ambus, P. Riso National Laboratory, P.O. Box 49, DK- 4000 Roskilde,  
fax: 45 46774160  
per.ambus@risoe.dk

Hauggaard-Nielsen, H. Plant Biology and Biogeochemistry Department, Riso National  
Laboratory, DK-4000 Roskilde  
fax: 45 46774160  
henrik.hauggaard-nielsen@risoe.dk

Jensen, E.S. Agroecology, Department of Agricultural Sciences, KVL,  
DK-2630 Agrovej 10  
fax: 4535282175  
esjensen@post12.tele.dk

## **EGYPT**

Abo- Hegazi, A.M.T. Radiobiology Department, Plant Breeding and Genetic Unit,  
Nuclear Research Centre, Atomic Energy Authority, P.O. Box 13759,  
Abu Zaabal, Cairo  
fax: 202 2911986

El-Kholi, A.F. Soil and Water Research Dept., Atomic Energy Authority,  
P.O. Box 13759, Abu Zabaal, Cairo  
fax: 202 2876031  
aeaegypt@facu.eun.eg

El-Motaium, R. Plant Research Department, Nuclear Research Center, PO Box 13759,  
Atomic Energy Authority, Abu Zaabal, Cairo  
rawia@@dns.claes.sci.eg

Galal, Y.G.M. Department of Soil and Water Resources, Nuclear Research Centre,  
P.O. Box 13759, Abu Zaabal, Cairo  
fax: 2022876031  
hegazi\_1@yahoo.com

Kamel, N. Radiation Protection Department, Nuclear Research Centre,  
Atomic Energy Authority, P.O. Box 13759, Abu Zaabal, Cairo  
fax: 202 2730261

Safwat, M.S.A. Department of Agricultural Microbiology, Faculty of Agriculture,  
Minia University, El-Minia  
fax: 02 5254898  
msasafwat@frcu.eun.eg

Thabet, E.M.A. Plant Research Department, Nuclear Research Centre,  
Atomic Energy Authority,  
P.O. Box 13759, Abu Zaabal, Cairo  
fax: 202 2911986

## **ESTONIA**

Parnik, T. Institute of Experimental Biology, Estonian Agricultural University,  
Instituudi tee 11, Harku 76902, Harjumaa  
fax: 372 6506091  
tiit@ebi.ee

## **ETHIOPIA**

Erkossa, T. Debre Zeit Agricultural Research Centre, EARO, P.O. Box 32,  
Debre Zeit  
fax: 2511 33 80 61  
dzarc@telecom.net.et

## **FRANCE**

Echevarria, G.F. ENSAIA-INPL, 2, Avenue de la Forêt de Haye, B.P. No. 172,  
F-54505 Vandoeuvre les Nancy  
fax: 33383595791  
echevarr@ensaia.inpl-nancy.fr

Fardeau, J.C. Departement Environnement – Agronomie,  
Institut national de la Recherche agronomique, Route de Saint-Lyr,  
F-78000 Versailles  
fax: 33 1 30833648  
fardeau@versailles.inra.fr

Morel, C.L.A. INRA Agronomie, BP 8, 1F-33883 Villenave d'Ornon Cedex  
fax: 33 5 56 84 30 54  
morel@bordeaux.inra.fr

Naulet, N.A.J.V. University of Nantes Faculté des Sciences et des Techniques,  
LAIEM 2, Rue de la Moussinière, 44322 Nantes Cedex 3  
fax: 33251125712  
norbert.naulet@chimbio.univ-nantes.fr

Recous, S. INRA, Unité d'Agronomie, F-02007 Laon  
sylvie.recous@laon.inra.fr

## **GERMANY**

Anisimova, M.A. Institute of Plant Nutrition and Soil Science, Bundesallee 50,  
D-38116 Braunschweig  
fax: 490531596377  
nissimova@yahoo.com

Hilkert, A.W.	Finnigan MAT GmbH, Barkhausenstrasse 2, Postfach 140 162, D-28197 Bremen fax: 00494215493396 101575,444@compuserve.com
Isermann, K.	Bureau for Sustainable Agriculture, Heinrich-von-Kleist-Str. 4, D-67374 Hanhofen fax: 6344 937264 isermann.bnla@t-online.de
Juchelka, D.G.	Finnigan MAT GmbH, Barkhausenstrasse 2, Postfach 140 162, D-28197 Bremen fax: 00494215493396 juchelka@finnigan.de
Oesselmann, J.	Finnigan MAT GmbH, Barkhausenstrasse 2, Postfach 140 162, D-28197 Bremen fax: 00494215493395 101511,417@compuserve.com
Schnug, E.	Institute of Plant Nutrition and Soil Science (FAL), Bundesalle 50 D-38116 Braunschweig fax: 49531596377 ewald.schnug@fal.de
Sparovek, G.	Institut fuer Pflanzenernaehrung und Bodenkunde, Bundesallee 50, FAL-PM D-38116 Braunschweig fax: 4953159677 gerd.sparover@fal.de

## **GHANA**

Asare, D.K.	Biotechnology and Nuclear Agriculture Institute (BNARI), P.O. Box LG80, Legon-Accra bnargaec@ghana.com
-------------	--------------------------------------------------------------------------------------------------------------

## **HUNGARY**

Oncsik, M.B.	Irrigation Research Institute, Szabadsag u. 2, H-5540 Szarvas fax: 3666 311178 oncsik@oki.ince.hu
Tetenyi, P.	Institute of Isotope and Chemistry, P.O.B. 77, H-1525 Budapest fax: 361 392 2533 tetenyi@alpha0.iki.kfki.hu

## **INDIA**

Manjaiah, K.	Nuclear Research Laboratory, Indian Agricultural Research Institute, New Delhi 11012 fax: 91115711902 manjaiah@excite.com or manjaiah@iari.ernet.in
--------------	--------------------------------------------------------------------------------------------------------------------------------------------------------------

- Mishra, S.P. Nuclear Radiochemistry Laboratory, Department of Chemistry,  
Banaras Hindu University, Varanasi 221 005  
fax: 91542 317074
- Nongkynrih, P. Physics Department, North-Eastern Hill University, Shillong  
fax: 364 250076  
phlissn@hotmail.com
- Raja Rajan, A. Tamil Nadu Agricultural University, Coimbatore 641 003  
fax: 91 04 22 439023  
rajarajan17@hotmail.com
- Sachdev, M.S. Nuclear Research Laboratory, Indian Agricultural Research Institute,  
New Delhi 110 012  
fax: 91-11-5766420  
mssachdev@excite.com
- Saradha, R. Nuclear Agriculture and Biotechnology Division,  
Bhabha Atomic Research Centre, Mumbai 400 085  
fax: 9122 5505151  
headbtd@magnum.barc.ernet.in

## **INDONESIA**

- Hakim, N. Research Centre for Utilization of Nuclear Techniques,  
Andalas University, P.O. Box 87, Padang  
fax: 625140040  
nhakim@indosat.net.id
- Sisworo, E.L. Centre for Research and Development of Isotope and Radiation  
Technology, Jl. Cinere Ps. Jumat Kotak, Pos 7002 JKSKL,  
Jakarta 12070  
fax: 7691607

## **IRAQ**

- Ali, A.H. Agricultural and Biological Research Centre,  
Iraqi Atomic Energy Commission, P.O. Box 765, Baghdad  
fax: 9641 7181929
- Fahad, A.A. Agricultural and Biological Research Centre,  
Iraqi Atomic Energy Commission, P.O. Box 765, Baghdad  
fax: 9641 7181929
- Shihab, R.M. Agricultural and Biological Research Centre,  
Iraqi Atomic Energy Commission, P.O. Box 765, Baghdad  
fax: 9641 7181929

## **JORDAN**

- Ababneh, M. National Centre for Agricultural Research and Technology Transfer  
(NCARTT), P.O. Box 639 Bagia, Post Code 19381  
fax: 96264726099  
ababneh99@hotmail.com

Al-Kharabsheh, A.A.

Al-Balga Applied University, Al-salt 19117  
fax: 9625 3557349  
atefkh@bau.edu.jo

## **KAZAKHSTAN**

Pak, L.

Agriculture State University, 222/60 Rozybakiev Str, 480060 Almaty  
fax: 73272633356  
Ljubov@ieem.almaty.kz

## **LEBANON**

Atallah, T.

Faculty of Agricultural Sciences, Lebanese University,  
P.O. Box 13-5368, Beirut (Chourane)  
fax: 9611 785427  
theresea@lynx.net.lb

## **MADAGASCAR**

Rabeharisoa, L.

Laboratoire des Radio Isotopes, B.P. 3383, 101 Antananarivo  
lrabehar@refer.mg

## **MALAYSIA**

Abu Bakar, R.

Land Management Department, Universiti Putra Malaysia,  
43400 Serdang, Selangor  
fax: 603 89434419  
rosenani@agri.upm.edu.my

Darus, S.Z.

Land Management Department, Universiti Putra Malaysia,  
43400 Serdang, Selangor  
fax: 603 89434419  
zaauyah@agri.upm.edu.my

Zaharah, A.R.

Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM,  
Serdang, Selangor  
fax: 60389434419  
zaharah@agri.upm.edu.my

## **MEXICO**

Cano-García, M.A.

Instituto Nacional de Investigaciones Forestales, INIFAP,  
Manuel Doblado 1010, Centro 68000, Oaxaca  
fax: 95141708,  
canogama@cirps.inifap.conacyt.mx

## **MONGOLIA**

Burmaa, B.

Soil Microbiology Laboratory, Plant Science and Agricultural  
Research Institute, Darkhan  
fax: 9763727144  
hureelen@mongol.net

## **MOROCCO**

- Benmansour, M. CNESTEN 65, rue Tansift, Agdal, Rabat  
fax: 212 7711940  
Benmansour@cnestn.org.ma
- Cherkaoui El Moursli, R. Faculty of Sciences, University Mohammed V, BP 1014, Rabat  
fax: 212 7 77 89 73  
rcherkao@fsr.ac.ma
- Ismaili, M. Faculté des Sciences, Université Moulay Ismail, B.P. No. 4010  
BeniMhamed, Meknes  
fax: 212 5 53 68 08  
ismaili@aim.net.ma

## **MYANMAR**

- Wai Wai Thet Tin, Department of Biotechnology, Yangon Technological University,  
Gyogone, Yangon  
fax: 951 667602

## **NEPAL**

- Shrestha, R.K. Soil Science Division, Nepal Agricultural Research Council,  
Khumaltar, Lalitpur  
fax: 977 01 521197  
shrestha@infoclub.com.np

## **PAKISTAN**

- Alam, S.M. Nuclear Institute of Agriculture, Tandojam  
fax: 22335284  
niatjam@hyd.paknet.com.pk
- Iqbal, M.M. Nuclear Institute for Food and Agriculture, NIFA, P.O. Box 446,  
Tarnab, Peshawar  
fax: 92912964059262733  
nifa@paknet2.ptc.pk
- Khan, D. Soil and Plant Nutrition, Agricultural Research Institute,  
Tarnab 24330, Peshawar NWFP  
fax: 9291 9216529
- Naqvi, M. c/o Aliz Risk Management Services, 5 Amber Towers, 22 A Block 6,  
PECHS, Karachi

## **Philippines**

- Rosales, C. Philippine Nuclear Research Institute, Commonwealth Avenue,  
Diliman, Quezon City  
fax: 951646  
amsasti@dost.gov.ph



## **POLAND**

Gorczyca, Z. Faculty of Physics and Nuclear Techniques, University of Mining and Metallurgy, Al. Mickiewicza 30, PL-30-059 Krakow  
fax: 0048126340010  
gorczyca@novell.ftj.agh.edu.pl

## **ROMANIA**

Serdinescu, A. Research Institute for Viticulture and Enology, Valea Calugareasca, Bdal Republicei Nr. 108, Bl. BB2 Ap. 14, 2000 Pliesti

## **RUSSIAN FEDERATION**

Oulianenko, L.N. RIARAE, Russian Academy of Agricultural Science, Kievskoye Road, Obninsk Kaluga Region, RU-249020  
fax: 095 255 22 25  
ustev@kaluga.ru

Ratnikov, A.N. RIARAE, Russian Academy of Agricultural Science, Kievskoye Road, Obninsk Kaluga Region, RU-249020  
fax: 095 255 22 25  
ratnikov@obnisk.ru

## **SAUDI ARABIA**

Alhabeeb, A.S.I. National Agriculture and Water Research Centre, P.O. Box 17285, Riyadh-Code 11484  
fax: 4584979  
nrrc@agrwat.gov.sa

## **SRI LANKA**

Gunaratne, W.D.L. Research Station, Department of Export Agriculture, Matale-21000  
fax: 948388738  
resexag@sltnet.lk

Sangakkara, U.R. Faculty of Agriculture, University of Peradeniya, Peradeniya 20400  
fax: 948 232517  
sanga@ids.lk

Wanniarachchi, S.D. Department of Agricultural Chemistry, Faculty of Agriculture, University of Ruhuna, Kamburupitiya 81100  
fax: 9441 92384  
sudaswan@sltnet.lk

## **SWEDEN**

Kirchmann, H. Swedish University of Agricultural Sciences, Box 7014, SE-750 07 Uppsala  
fax: 4618 672795  
holger.kirchmann@mv.slu.se

## **SWITZERLAND**

- El-Hajj, G. Institute for Plant Sciences, (ETHZ), Eschikon 33, CH-8315 Lindau  
fax: 41 52 354 9119  
gaby.hajj@ipw.agrl.ethz.ch
- Gallet, A. Institute for Plant Sciences (ETHZ), Eschikon 33, CH-8315 Lindau  
fax: 41 52 354 9119  
anne.gallet@ipw.agrl.ethz.ch
- Schneider, M. ETH Zentrum LFW A2, Universitaetstrasse 2, CH-8092 Zürich  
fax: 41 1 632 1153  
manuel.schneider@ipw.agrl.ethz.ch
- Sinaj, S. Institute for Plant Sciences (ETHZ), Eschikon 33, CH-8315 Lindau  
fax: 410523549119  
sokrat.sinaj@ipw.agrl.ethz.ch

## **SYRIAN ARAB REPUBLIC**

- Janat, M.M. Department of Agriculture, Atomic Energy Commission,  
P.O. Box 6091, Damascus  
fax: 6112289

## **THAILAND**

- Jitpratug, W. Ganglen Operation and Maintenance Project 205, MOO 14 Srapatana,  
Aumphoe Kumpangsan, Nakornprathom 73180
- Mahisarakul, J. Nuclear Research in Agriculture Laboratory, Division of Agricultural  
Chemistry, Chatuchak, Bangkok 10900  
fax: (662)5797158  
Jittiwan@doa.go.th
- Pakkong, P. Applied Radiation and Isotopes Department, Faculty of Science,  
Kasetsart University, Chatuchak, Bangkok 10900  
fax: 662 579 5530  
fscipnp@nontri.ku.ac.th
- Phongpan, S. Nuclear Research in Agriculture Section, Division of Agricultural  
Chemistry, Department of Agriculture, Chatuchak, Bangkok 10900  
fax: (662)5797158 (662)5797158

## **TUNISIA**

- Mtimet, A. Direction des Sols, Ministère de l'Agriculture, 30 Rue Alain Savary,  
1002 Tunis  
fax: 2161718208  
dar.mtimet@planet.tn

## **TURKEY**

- Halitligil, M.B. Ankara Nukleer Tarim Ve, Hayvancilik Arastirma Merkezi,  
Istanbul Yolu 30 km, Saray-Ankara  
fax: 3128154307  
basri@taek.gov.tr

Kirda, C. Agriculture Structures and Irrigation Department, University of  
Cukurova, 01330 Adana  
fax: 90322 3386386  
ckirda@mail.cu.edu.tr

## **UGANDA**

Sessanga, S.M. Soil Science Department, Makerere University, P.O. Box 7062,  
Kampala  
fax: 25641 533574  
acss@starcom.co.ug

## **UNITED KINGDOM**

Walling, D.E. Department of Geography, Amory Building, University of Exeter,  
Rennes Drive, Exeter, Devon EX4 4RJ  
fax: 441392263345

## **UNITED STATES OF AMERICA**

Evett, S.R. Conservation and Production Research Laboratory, USDA-ARS,  
P.O. Drawer 10, 2300 Experiment Station Road, Bushland, TX 79012  
fax: 806 356 5750  
srevett@ag.gov

L'Annunziata, M.F. The Montague Group, P.O. Box 1471, Oceanside, CA 92051-1471  
fax: 760 439 5089  
lannunziata@compuserve.com

## **VENEZUELA**

Camacho, R.G. Departamento de Produccion Vegetal, Universidad Romulo, Gallegos,  
Ciudad Universitaria, San Jan de los Morros, 2301 Guarico  
fax: 58 46 312670  
rcamacho@gur.reacciun.ve

## **VIET NAM**

Hien, P. D. Viet Nam Atomic Energy Commission, 59 Ly Thuong Kiet Street,  
Hanoi  
fax: 008448266133  
Pdhen@netnam.org.vn

Hoang, Dac Luc Institute of Nuclear Science and Technique, Viet Nam Atomic Energy  
Commission, P.O. Box 5T-160, Hoang Quoc Viet Street, Cau Giay,  
Hanoi  
fax: 4 8363295  
hdluc@eudoramail.com

Luong, Thu Tra Centre for Nuclear Techniques, 217 Nguyen Trai. Dist. 1,  
Ho Chi Minh City  
fax: 84 8 8367361  
ttkhn@hcm.vnn.vn

## **ZAMBIA**

Malama, C. Misamfu Regional Research Centre, P.O. Box 410055, Kasama  
fax: 002604221135  
misamfu@zamnet.zm

## **CIEC**

Hera, C.I. International Scientific Centre of Fertilizers (CIEC),  
Spatarul Nic Milescu Str. 32, Buccharest Sect. 2 73354  
fax: 4012508942  
hera@valhalla.racal.ro

## **IAEA**

Chalk, P. Joint FAO/IAEA Division of Nuclear Techniques in Food and  
Agriculture, International Atomic Energy Agency, P.O. Box 100,  
A-1400 Vienna  
fax: 43126007  
p.m.chalk@iaea.org

Hardarson, G. Agency's Laboratories, Seibersdorf, International Atomic Energy  
Agency, P.O. Box 100, A-1400 Vienna  
fax: 431260028222  
G.Hardarson@iaea.org

Heng, L.S. Agency's Laboratory, Seibersdorf, International Atomic Energy  
Agency, P.O. Box 100, A-1400 Vienna  
fax: 431260028222

Hood, R. Agency's Laboratories, Seibersdorf, International Atomic Energy  
Agency, P.O. Box 100, A-1400 Vienna  
fax: 431260028222  
R.Hood@iaea.org

Keerthisinghe, G. Joint FAO/IAEA Division of Nuclear Techniques in Food and  
Agriculture, International Atomic Energy Agency, P.O. Box 100,  
A-1400 Vienna  
fax: 43126007  
G.Keerthisinghe@iaea.org

Moutonnet, P. Joint FAO/IAEA Division of Nuclear Techniques in Food and  
Agriculture, International Atomic Energy Agency, P.O. Box 100,  
A-1400 Vienna  
fax: 43126007  
P.Moutonnet@iaea.org

Salema, P. Joint FAO/IAEA Division of Nuclear Techniques in Food and  
Agriculture, International Atomic Energy Agency, P.O. Box 100,  
A-1400 Vienna  
fax: 43126007  
m.p.salema@iaea.org

Zapata, F.

Joint FAO/IAEA Division of Nuclear Techniques in Food and  
Agriculture, International Atomic Energy Agency, P.O. Box 100,  
A-1400 Vienna  
fax: 43126007  
f.zapata@iaea.org

## **IUSS**

Blum, W.E.H.

University of Agricultural Sciences, Gregor Mendel Strasse 33,  
A-1180 Vienna  
fax: 431 47891 10

## **UNIDO**

Sanchez Osuna, M.

Cleaner Production and Environmental Management Branch, UNIDO,  
P.O. Box 300, A-1400 Vienna  
fax: 431 2692669



