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FOREWORD

This publication deals with the topic of nitrogen management in agro-ecosystems. Nitrogen (N) is an essential plant nutrient, and N deficiency severely restricts crop yields in most cultivated soils. Therefore, substantial N inputs are required for optimum plant growth and adequate food, feed and fibre production. Developing countries use more than 55 million metric tons (t) of N fertilizers at an estimated value of US $16 billion annually, of which approximately 2 million t are used in Africa, 5 in Latin America and 50 in Asia. It is estimated that adequate production of food (in particular cereals) for present and future populations will not be achieved without external inputs of fertilizer N. However, management practices involving fertilizer N should be efficient in order to optimize crop production while minimizing adverse effects on the environment. Moreover, the use of alternative N sources such as organic residues and biological nitrogen fixation should be increased within the context of integrated soil fertility management to ensure food security in areas of the world where fertilizer N is too expensive or simply not available. At present, legumes such as soybean, common bean, groundnuts, chickpeas, cowpeas, etc., are fixing approximately 11 million t of N in developing countries.

This publication covers, concisely and comprehensively, key topics dealing with the utilization of all sources of N in farming systems, in particular to demonstrate to scientists in developing countries how isotopic tracer technologies can be used in research to improve overall N use efficiency in agricultural systems while increasing crop yields in a sustainable manner, i.e. conserving the natural resource base and protecting the environment. It is a timely publication; increasing attention is being paid to N management in food production, energy consumption and environmental protection.

The subject matter is covered in four chapters, starting with an introduction to N management in agricultural systems (Chapter 1). The following three chapters cover the main sources of N in crop production, namely, mineral N fertilizers (Chapter 2), biologically fixed N (Chapter 3) and organic N sources (Chapter 4). Within each of these latter three chapters, the theoretical basis and applications of stable isotope ^{15}N tracer techniques to measure N process rates and N balance from different N sources in various cropping systems are elaborated. The publication is completed with a brief conclusion.

This publication is the fourth in the IAEA Training Course Series produced by the Soil and Water Management and Crop Nutrition Sub-Programme. It was conceived as a technically oriented document for a target audience comprising soil and environmental scientists and technicians, agronomists, ecologists, extension workers, and upper-level undergraduate and graduate students in these disciplines, staff of non-governmental organizations (NGOs) and other stakeholders involved in sustainable agricultural development at local, national, regional and international levels.

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EDITORIAL NOTE

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CHAPTER 1

INTRODUCTION TO NITROGEN MANAGEMENT IN AGRICULTURAL SYSTEMS

Felipe ZAPATA
Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture,
International Atomic Energy Agency,
Vienna

Abstract

Background information is provided on nitrogen as an essential plant nutrient and on relevant aspects of the global nitrogen cycle, with a brief historical account of the use of nitrogen sources in agriculture. Past and current nitrogen research work is described, in particular that in the Joint FAO/IAEA Programme using isotopic tracers. The role of nitrogen in sustainable intensification of agricultural production is discussed.

1.1. NITROGEN AND PLANT NUTRITION

All forms of life on Earth require energy, nutrition and water. Water, light, oxygen, carbon dioxide and numerous mineral elements are needed for plant growth and adequate production of food and fibre. The element nitrogen (N) is essential for the normal growth of plants. All vital biological processes are related to the existence of functional plasma, of which N is a basic constituent (proteins, nucleic acids). Nitrogen is also a basic constituent of many other compounds of primary physiological importance to plant metabolism, such as chlorophyll, nucleotides, proteins, alkaloids, enzymes, hormones and vitamins [1].

Nitrogen is a nutrient required by plants in comparatively larger amounts than are other soil-borne elements; endogenous application to crops often results in yield improvement. Many legumes and certain other species can obtain N from the atmosphere, but most species obtain it only from the soil. In agricultural systems, N is obtained from the soil through mineralization of soil organic matter and from external sources, both organic and inorganic. For an optimal yield, the N supply must be available according to the needs of the plant, matching its pattern and total amount.

Nitrogen deficiency may exert pronounced adverse effects on crop development and yield. Growth is stunted, and leaves become chlorotic because lack of N limits the synthesis of proteins and chlorophyll. Lack of chlorophyll inhibits the capacity of the plant to assimilate CO_2 and synthesize carbohydrates, leading to poor and premature flowering and fructification, with shortening of the growth cycle. Nitrogen deficient plants respond quickly to the addition of N fertilizers if applied in a timely manner and properly. However, adverse effects on annual plants caused by early-stage lack of N cannot usually be corrected by late application of N [1, 2].

The presence of N in excess promotes development of the aerial organs with relatively poor root growth. Synthesis of proteins and formation of new tissues are stimulated, and thus carbohydrates of high molecular weight are synthesized in insufficient amounts, resulting in abundant dark green (high chlorophyll) tissues of soft consistency. This increases the risk of lodging, and reduces the plant's resistance to harsh climatic conditions and to foliar diseases

and insect predation. It also extends the growth cycle, delaying maturity, and often reduces the quality of the harvestable products [2].

1.2. THE NITROGEN CYCLE

A detailed examination of the global N cycle as it affects natural and agricultural systems is beyond the scope of this paper. Many books and treatises are available elsewhere on this subject (e.g., Refs [3–8]). Nevertheless, it is worth considering the following issues of the global N cycle resulting from human activities.

1.2.1. Carbon-cycle coupling

Carbon, like N, plays a key role in the vast biochemical cycles of life. The carbon and N cycles are continuously being reshaped by human activity, including changes in land use and farming/agricultural practices. It is advisable to study the dynamics/cycling of both elements together to fully understand the cycling processes involved, their significance and interactions, including effects on soil biodiversity. Equally important are the long term effects of land use and tillage systems on N cycling, carbon accumulation and carbon sequestration potential [9–11]. With respect to soil biodiversity, due attention should be paid to interactions of the components of the agro-ecosystem with a focus on human activity, as well as to the complex food webs and functions of the system and the less tangible attributes of landscape diversity [8, 12–15].

Nitrogen and carbon are common and abundant in nature. Nitrogen comprises 78% of the atmosphere, almost all of it as the relatively inert molecule N_2; it is frequently a limiting factor for plant production.

1.2.2. Nitrogen fixation

Elemental N (N_2) cannot be metabolized by eukaryotes. Nitrogen becomes biologically active when it is fixed or bound, i.e. incorporated into other molecules (primarily ammonium (NH_4^+) and nitrate (NO_3^-)). Fixed N flows through the food web (plant–animal–humans/predators). This fixation process distinguishes the N cycle from the carbon cycle.

From a global environmental point of view, there is a serious concern about the growing fixed-N glut. Estimates of inputs from several sources (fossil fuels, mineral fertilizers, biomass burning, land clearing, etc.) caused by recent and uncontrolled human activity are presented in Table I; nitrogen fixed by human activity now exceeds all terrestrial natural processes combined.

It is estimated that agriculture accounts for by far the largest fraction (some 86%) of the total human-released fixed N [16].

1.2.3. Soil nitrogen

As there are no minerals containing N in soil, reserves of N depend on the soil organic matter content. Thus, N cycling in soil is closely related to organic matter turnover. Micro-organisms are responsible for soil-N transformations, which play a key role in determining the availability of N for plant growth and crop production [17–20]. Therefore, the knowledge of the quality of soil organic matter and understanding of these N-cycling processes are of utmost importance with respect to agricultural systems for the balanced and proper

management of external sources of N in the forms of mineral and organic fertilizers and biologically fixed N [21].

TABLE I. GLOBAL NITROGEN CYCLE

(estimates of annual releases of fixed nitrogen (terrestrial sources only) caused by human activity [12, 15])

Source	Million metric tons
Fertilizer	80
N_2-fixing crops	40
Fossil fuels	20
Biomass burning	40
Wetland drainage	10
Land clearing	20
Total anthropogenic sources	210
Total natural sources of fixed-N production	140

Mineral N is highly mobile in the soil and, if not taken up by plant roots and microbes, it can be lost through leaching (downward movement within the soil profile beyond the rooting zone) and gaseous emissions, mainly by denitrification and volatilization, creating environmental hazards [22, 23].

Changes in the N cycle associated with excessive soil-N loading can have detrimental effects on terrestrial and aquatic ecosystems, such as eutrophication, algal blooms and dead zones (oxygen depleted coastal waters) [24–26] as well as surface water and groundwater contamination [27], greenhouse gas emissions [28], etc. Also, other ecological impacts (changes in the structure of ecosystems and biodiversity) as well as economic impacts may result from disruptions in the global N cycle [15, 25, 29].

Similarly, the use of reactive (fixed) N affects human health both in positive and in negative ways, depending on the rates of reactive N used in the ecosystem. Negative health effects of highly reactive N are both direct (pollution of air and water) and indirect (ecological feedback to disease). It is possible to reduce environmental and associated health problems by changing the N cycle [30].

1.2.4. Mitigation efforts

Global efforts to address N related issues in an integrated manner have been and are currently being coordinated under the International Nitrogen Initiative. For details on this and related projects/activities, the reader is referred to Ref. [31]. The UN Environment Programme is leading a Global Plan of Action for the protection of marine environments from adverse land-based activities, including the impact of all N sources [25, 26, 32].

With regard to N cycles within agro-ecosystems or specific cropping/farming systems, extensive reviews are available [17–19, 33–37]. They have been defined mainly for the purpose of establishing N balances (inputs/outputs) and/or modelling. Moreover, OECD countries have been involved recently in the assessment of the impacts of agricultural systems

on the environment, in particular the development and use of environmental indicators at the farm level. This work focuses also on nutrient accounting in soil, water and food products [13, 14]. Mishima [38] and Hatano et al. [39] in Japan and southern China have made case studies of N accounting at the national level.

1.3. HISTORY OF THE USE OF SOURCES OF NITROGEN IN AGRICULTURE

In many natural ecosystems such as the tropical rain forests of the Amazon and Central Africa, a closed nutrient cycle exists. In the case of N, balance studies have shown that losses from soil due to uptake, leaching, erosion, denitrification, etc., are apparently replenished to varying extents by biological N_2-fixation processes. When agriculture is introduced into these systems, this nutrient cycle is broken and the normal supply of nutrients for any agricultural activity comes mainly from the soil. Additional nutrients must be supplied as external inputs to maintain crop production. In ancient times, the only way to provide such nutrients was through the addition of organic materials such as human, animal and crop wastes to the soil. There are many examples of the disappearance of whole civilizations due to diminished soil fertility and productivity [2].

During the first half of the nineteenth century, the development of the concept of plant nutrition, supported by chemistry (agro-chemistry), led to the birth of the fertilizer industry. Initially, natural products (potash and rock phosphate deposits, guano, sodium nitrate) and industrial by-products (basic slags, ammonium sulphate) were utilized. Only during the first two decades of the twentieth century were economic means of synthesizing ammonia from atmospheric nitrogen (Haber–Bosch process) developed, enabling the manufacture of N fertilizers. Since then, N fertilizer production has continuously increased, N fertilizer being based mainly on the synthesis of ammonia using hydrogen from hydrocarbons. Today, the world's most common and cheapest N fertilizer is urea, as a result of the implementation of modern industrial technologies and related economies of scale [40].

During the past three decades, agricultural intensification through the use of high yielding varieties, chemical fertilizers and pesticides, irrigation and mechanization — the Green Revolution — has been responsible for significant increases in grain production in some developing countries. For instance, between 1961 and 1965 the world's cereal production area averaged 677 million ha and annual cereal production 988 million t. The averages between 1994 and 1995 were 699 million ha and 1,970 million t, i.e. increases of 3% in area and of 99% in production; fertilizers, among other agricultural practices, contributed significantly to these increases. The FAO Fertilizer Programme that was operational worldwide concluded that fertilizers were responsible for some 55% of the increase in yields in developing countries between 1965 and 1976. Fertilizers, after land and water, are probably the most important input leading to increased yields [41–44]. Considering that mineral fertilizers contribute about 40% of the N taken up by crops and that crops provide about 75% of the protein N consumed by humans, it is estimated that approximately one third of this protein depends on fertilizer N [45, 46]. For more information on aspects related to the fertilizer industry, fertilizer use in agriculture and its impact on the environment, including historical details, the reader is referred to Refs [40, 47, 48].

During the 1974 oil crisis, the shortage of mineral fertilizers and their high prices led to the initiation worldwide of several research programmes on biological N_2 fixation in legumes. However, in a recent FAO consultation it was found that there are limitations to the adoption and use of these technologies by farmers in developing countries, including research gaps in some topics. It was also concluded that effort must be made to promote the expanded use of

biological N_2-fixation-based farming systems to help small scale, resource poor farmers to improve the food security of developing countries. Biological N_2 fixation is considered to be a viable, cost effective alternative or complementary solution to the increased use of industrially manufactured N fertilizers [49, 50].

Urbanization of modern societies is leading to increased production of human, animal and industrial wastes that require disposal, creating environmental problems mainly in developed industrialized countries but also in some regions of the developing world. If present trends continue, the global production of wastes will increase fivefold by 2025, increasing pollution and associated health risks, especially in developing countries [24, 51]. Where it can be done safely, nutrients contained in organic wastes should be recycled for economic and environmental reasons [52]. This is, in fact, practiced in organic agriculture. However, in most developed countries and in some developing countries, the main problem is the disposal of huge amounts of manure and slurry from large production units — concentrated animal feeding operations (CAFOs) — in confined areas. The disposal of such wastes is increasingly controlled by legislation in most developed countries. However, demands for meat in growing populations are high, and livestock intensification and associated environmental problems are being shifted to developing countries.

For agricultural production to keep pace with population growth and increasing food demands, intensification will be required with increasing dependence on fertilizer application worldwide. This trend is likely to be particularly marked in developing countries. However, it is important to note that such intensification may also have detrimental impacts on the environment, causing economic losses in both developed and developing countries. For detailed information on this, see Section 1.1.6.

1.4. TRENDS IN NITROGEN RESEARCH

The general trends in agricultural development outlined above have strongly influenced research on N. The goal of much of this research has been to improve overall N use efficiency in agricultural systems. As N cycling in soil is closely related to soil organic matter turnover, traditional studies focused on the predominant processes affecting the dynamics of soil organic matter, in particular mineralization/immobilization turnover (MIT). Currently, studies are carried out on particular fractions, which are separated by physical or chemical means in order to gain a better understanding of N dynamics in soils for use, where appropriate, as indicators of soil quality [53, 54].

Fertilizer-N research and development has been concerned mainly with agronomic and economic aspects related to crop responses to applications in specific countries/locations. Because chemical fertilizers are applied to a number of crops grown under diverse environments and management practices, the fertilizer-N use efficiency by a given plant (species and variety) depends on a number of local factors (soil, climate, cropping/farming system, management practices, etc.) and their interactions [42, 43, 55–58].

Under the FAO Fertilizer Programme, which operated in many developing countries, field trials and demonstrations were conducted in several locations per country with the objective of maximizing economic yields from given rates of fertilizer application. Since such fertilizer studies produce site specific recommendations, they are of a continuing nature due to changes in the various influencing factors and multiple interactions among them. The data obtained are also useful for socioeconomic studies of fertilizer use and adoption, to define policies and guidelines at regional and national levels [41, 42, 44]. Moreover, extensive work has been

conducted by researchers in both the developed and developing world on a wide range of crops in various environments, in order to identify best management practices to increase efficiency of use of applied fertilizer N, i.e. recovery by the crop, on research stations as well as on farms.

Follow-up research included studies on the fate of applied fertilizer within cropping sequences in terms of uptake by the plant, residual in the soil (accounted for) and lost from the system (unaccounted for).

Increasing concerns over potential environmental hazards and ecological impacts have led to the development of research areas related to mechanistic studies and measurement methods to control/mitigate N losses [27, 28]. Recent efforts to develop innovative technologies and best management practices with fertilizer N in cropping systems include improved rapid methods for N monitoring and provision of advice for fertilizer N recommendations, including precision-farming techniques and modelling tools [36, 37, 59].

Recent international meetings have identified the need for more research on specific aspects of biological N_2 fixation oriented to the development and introduction of viable and cost effective technologies to farming systems, considering the needs and constraints of small scale, resource poor farmers in developing countries [49]. Increasing research attention has been paid to the characterization of organic residues, using standardized methods and the establishment of databases on quality parameters of important locally available products to better predict their nutrient supply with time in order to match crop demands and their effect on soil properties [60–65]. Scope and limitations in the management of organic matter in tropical soils [66] and effects of organo-mineral combinations (mixtures of organic materials with mineral fertilizers) on soils and crops and their fate in cropping systems have been assessed; however, more studies are needed [21, 67–69].

Currently, investigations are being conducted with a more integrated approach on the use of locally available sources of N at the cropping system level. These studies focus on N cycling/dynamics and the assessment of best means of management of the N sources, with the ultimate goal of improving overall N use efficiency in agricultural systems [31, 67, 70–72].

1.5. NITROGEN RESEARCH IN THE JOINT FAO/IAEA PROGRAMME

Since its creation in 1964, the Food and Agriculture Organization of the United Nations (FAO) and the International Atomic Energy Agency (IAEA) have, through their Joint FAO/IAEA Division in Vienna and the Agriculture Laboratory in Seibersdorf, Austria, promoted the development and transfer of nuclear technologies to help Member States establish better conditions for sustainable crop and livestock production systems while ensuring food security. Technology transfer is achieved through the implementation of various international network mechanisms such as Coordinated Research Projects (CRPs) and Technical Cooperation Projects (TCPs). In support of these projects, fellowships and training courses are also offered [73]. The Joint FAO/IAEA Programme has closely followed the development of new technologies and trends in agricultural production, including the Green Revolution in the 1970s.

In view of the essential role that soil and fertilizer N play in maintaining and increasing crop yields in modern intensive agriculture, the stable isotope ^{15}N has been widely used in several projects of the FAO/IAEA Programme as a tracer to quantitatively determine amounts and

movement in plants and soil of N derived from applied fertilizers. Early CRPs (1964–1985) focused on maximizing the efficiency of fertilizer use by major food crops including those grown in multiple cropping systems (Table II).

TABLE II. INTERNATIONAL RESEARCH PROJECTS ON FERTILIZER USE EFFICIENCY COORDINATED BY THE JOINT FAO/IAEA PROGRAMME

Project title	Duration
Rice fertilization	1962–1968
Fertilizer management practices for maize: Results from experiments with isotopes	1964–1968
Isotope studies on wheat fertilization	1968–1972
Root activity pattern of tree crops	1967–1972
Isotope studies on rice fertilization	1970–1974
Use of isotopes for study of fertilizer utilization by legume crops	1972–1977
Isotope aided micronutrient studies in rice production with special reference to zinc deficiency	1974–1979
Soil N as fertilizer or pollutant	1975–1983
Nuclear techniques in the development of fertilizer practices for multiple cropping systems	1980–1985

Most of the studies focused on the development of the most efficient fertilizer-N management practices for grain crops [55]. Furthermore, the environmental behaviour of soil and fertilizer N was studied with emphasis on the fate of the applied N and monitoring of its residues in the soil under various cropping systems and environments [34, 35, 74].

Some 30 years ago, in connection with the oil crisis of 1974 and associated increases in fertilizer prices, projects on biological N_2 fixation (BNF) were initiated with a CRP on ^{15}N methodology for measuring BNF in grain legume crops. Other projects followed, with quantification of BNF in forage, pasture and tree legumes and in *Azolla*, in a range of environments (Table III). Recent projects have emphasized enhancement of BNF in selected grain legume crops through genetic improvement [75].

TABLE III. INTERNATIONAL RESEARCH PROJECTS ON BIOLOGICAL NITROGEN FIXATION COORDINATED BY THE JOINT FAO/IAEA PROGRAMME

Project title	Duration
Grain legumes	1979–1983
Multiple cropping	1980–1985
Pasture and forage legumes	1983–1988
Azolla and blue-green algae	1984–1989
Common bean in Latin America	1986–1991
Grain legumes in Asia	1987–1994
Tree legumes	1989–1995
Rhizobial ecology	1992–1997

TABLE IV. FAO/IAEA INTERNATIONAL COORDINATED RESEARCH PROJECTS (CRPS) AND TECHNICAL CO-OPERATION PROJECTS (TCPs) ON INTEGRATED APPROACHES TO SOIL, WATER AND NUTRIENT MANAGEMENT

Project title	Duration
Plant nutrient and water balance methods (cereal-legume or fallow-cereal) in Middle East (TCP)	1991–1994
Fertilizer nitrogen in irrigated wheat (CRP)	1994–1998
Water balance and fertigation in West Asia (TCP)	1995–2000
Agronomic effectiveness of phosphate sources, in particular phosphate rocks (CRP)	1995–2000
Use of irradiated sewage sludge in cropland (CRP)	1995–2000
Management of crop residues in cereal-legume crop rotations (CRP)	1995–2000
Plant nutrition, soil and water management in Latin America (TCP)	1996–2001
Increased crop production in rainfed cropping systems in arid and semi-arid areas (CRP)	1998–2003
Development of agro-forestry systems (CRP)	1998–2005
Sustainable crop production systems in tropical acid soils of the savannas of Africa and Latin America (CRP)	1999–2004
Sustainable rice-wheat cropping systems in Asia (CRP)	2001–2005
Fertigation for improved crop production in Europe (TCP)	2001–2003
Restoration of soil fertility and sustenance of agricultural productivity in Asia (TCP)	2001–2004
Combating desertification in the Sahel (TCP)	2001–2004

In 1995, an external review recommended a new strategic objective for the Soils Sub-Programme of the Joint FAO/IAEA Division, i.e. to develop and promote the adoption of nuclear based technologies for optimizing soil, water and nutrient management in well defined cropping systems and agro-ecological zones, to support intensification of crop production and preservation of the natural resource base [72, 76]. Since then, integrated approaches to soil, water and nutrient management have been implemented in CRPs and TCPs as shown in Table IV.

Current studies conducted under regional TCPs and CRPs include those on rainfed cropping systems in semi-arid areas, cropping systems of the humid/subhumid savannas of Africa and Latin America, rice-wheat cropping systems in Asia, and agro-forestry systems worldwide [73].

1.6. NEED FOR SUSTAINABLE INTENSIFICATION OF AGRICULTURE

The global population, currently 6000 million, is expected to reach 8000 million by 2020 and most of that increase will occur in the developing world [77, 78]. In addition, increased urbanization and industrialization of the rapidly growing population will result not only in greater but also changing food demands. Pressure on land to increase productivity is causing degradation of soil worldwide and expansion of agriculture into marginal areas.

Approximately 50% of potentially arable land is currently under cultivation. A further 2000 million ha (23% of agricultural land) are already degraded; degradation continues through a wide range of processes, related mainly to mismanagement [24, 25, 79, 80]. The livelihood and economic well-being and nutritional status of over a billion people are already affected by land degradation [80–82]. Furthermore, in South America and sub-Saharan Africa, a predicted 12% increase of cultivated land by 2030 will be mostly in marginal areas [79, 83].

In many developing countries, continuous cultivation with inappropriate farming practices has resulted in severe depletion of nutrients and soil organic matter, seriously threatening agricultural production. Sanchez et al. [84] reported that annual losses of nutrients in Africa were equivalent to 7.9 Mt of NPK, six times its annual fertilizer consumption. Henao and Baanante [85] estimated high rates of nutrient loss from agricultural soils of Africa as a whole, and Pieri [86] similarly analysed West African savannah soils.

Depletion of soil organic matter in tropical regions can be as high as 70% as a result of cultivation for 10 years [87]. As the reservoir of nutrients and energy that drives biological processes involved in nutrient cycling (and availability), soil organic matter is a key factor in maintaining long term fertility. It also has a profound influence on soil chemical (cation exchange capacity, buffering of soil pH, chelation of metals, etc.) and physical (stabilization of soil structure, water retention, etc.) properties [53]. Agricultural production cannot be sustained if nutrients removed during cropping are not replenished or if appropriate agricultural practices are not implemented to maintain and/or increase soil organic matter.

Competition for scarce water resources is another major constraint to increasing food production in developing countries. Agriculture is by far the largest user of water, accounting for around 70% of withdrawals worldwide and 90% in low income developing countries [88]. Moreover, rapidly growing municipal and industrial demands for water in developing countries will increase scarcity for agriculture, and with continued slowdown in water investments could be a serious threat to future growth in food production [89].

Against this global background, several developing countries will face major challenges in achieving sustainable food security. A number of constraints and limitations are in play, such as available per capita land area, severe scarcity of freshwater resources, particular socio-economic conditions of the agriculture sector, and internal structures and conflicts [81, 90]. Thus, the key challenges are to develop global capacity to produce adequate food in a sustainable manner, and in particular to improve the ability of poorer countries to produce agricultural commodities (food and cash crops), i.e. not only to increase their food supply but also to generate income and employment through growth of the agricultural sector. Both natural resources (land, water, forests, fisheries, etc.) and agricultural inputs (water, fertilizer, pesticides, seeds, mechanization, energy and technology) are essential factors determining increases in crop production. Degradation of natural resources undermines production capacity while availability of, and access to, agricultural inputs influences actual productivity and production levels. Current knowledge for achieving sustainable crop intensification through measures that both optimize the use of external inputs and conserve natural resources within diverse cropping systems and agro-ecological zones is inadequate. In order to increase intensification and diversification of agricultural production systems towards supporting productivity gains and income generation, innovative sustainable technologies will have to be developed, pilot tested and transferred in a relatively short time. Appropriate policies and economic incentives will also be required for use and adoption of viable, cost effective technologies [76, 91].

Therefore, it is postulated that a more holistic and integrated approach to the management of the components of cropping systems in main agro-ecological regions will be required to develop innovative technologies for sustainable crop production. In the case of N, there is a need to gain refined information on N-cycling processes and to assess the value of the crop/soil/fertilizer management practices designed to improve overall N use efficiency of the agro-ecosystem, with the ultimate goal of enhancing sustainable intensification of agricultural production while conserving the natural resource base [31, 72].

7. THESE GUIDELINES

From the above and from the references and further reading, it is clear that a wealth of information exists on many aspects related to N cycling in agro-ecosystems. This publication attempts to cover concisely but comprehensively the key topics dealing with the utilization of all sources of N in farming systems, i.e. mineral fertilizer N (Chapter 2), biological nitrogen fixation (Chapter 3) and organic sources (Chapter 4), and in particular to demonstrate to scientists from developing countries how isotope tracer technologies can be used in N research to improve overall N use efficiency in agricultural systems while increasing crop yields in a sustainable manner, i.e. conserving the natural resource base and protecting the environment.

These guidelines are conceived as a technically oriented document for a target audience comprising soil and environmental scientists and technicians, agronomists, ecologists, extension workers, and upper level undergraduate and graduate students in these disciplines, as well as staff of non-governmental organizations (NGOs) and other stakeholders involved in sustainable agricultural development at local, national, regional and international levels.

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CHAPTER 2

USE OF TRACER TECHNOLOGY IN MINERAL FERTILIZER N MANAGEMENT

Oswald VAN CLEEMPUT
Ghent University,
Ghent,
Belgium

Felipe ZAPATA
Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture,
International Atomic Energy Agency,
Vienna

Bernard VANLAUWE
Tropical Soil Biology and Fertility Institute of CIAT,
Nairobi,
Kenya

This chapter provides background information on fertilizer nitrogen (N) consumption and production (2.1) and estimates of future requirements, followed by methods for the measurement of fertilizer N use efficiency (2.2). Aspects relevant to the use of fertilizer N in agricultural production systems, such as factors affecting its efficiency and loss (2.3), and interactions with other N sources and soil testing for providing fertilizer recommendations, are discussed, as are approaches/strategies to improve fertilizer N efficiency (2.4), with particular emphasis on the use of isotopic tracers.

2.1. MINERAL NITROGEN FERTILIZER MANAGEMENT

2.1.1. INTRODUCTION

Nitrogen (N) is the most widely applied fertilizer because it is usually considered the main nutrient limiting factor in most agricultural systems. In terms of terminology, a distinction is made between N fertilizer and organic N inputs, thereby avoiding a term such as "organic fertilizer" as, per definition, the term "fertilizer" applies to materials that contain at least 5% of one or more of the three primary nutrients — N, phosphorus (P) and potassium (K) — in available form [1].

The key role that fertilizer N has played in increasing crop yields, in particular of cereals, is widely recognized. For instance, some 10 kg of cereal grain per kg of fertilizer nutrient N+P+K is considered a typical fertilizer–response ratio [2]. Nonetheless, there are situations where excessive or improper use of fertilizer N can cause severe environmental damage such as eutrophication of surface waters and contamination of groundwater [3–10].

Trends in crop production and fertilizer use have been examined in many countries. Studies by Bock and Hergert [11] and by Marks [12] in the United States of America and the United Kingdom, respectively, showed that the N removed from fields in harvested corn grain has tripled over the past 40 years, whereas the rate of fertilizer N increased more than fifteenfold in the same period. A similar study was made in China [13]. Selected data below provide an understanding of the role of fertilizer N in agricultural production.

2.1.1.1. Fertilizer nitrogen consumption, production and future requirements

In this section, past and current consumption and production of fertilizer N are briefly assessed both at regional and at global levels. Moreover, a forecast of future fertilizer N requirements is presented. Most of the information is drawn from FAO and IFA statistical data. For detailed information, the reader is referred to the FAO and IFA web sites [14–17].

There are three forms of mineral N in fertilizers: nitrates supply NO_3^- ions, ammonium salts supply NH_4^+ ions, and amides contain N in $–NH_2$ form or forms derived from it. Plants take up ammonium and nitrate ions, and, except in very acid soils and anaerobic conditions, ammonium N is quickly converted to nitrate N. Amides (e.g. urea) are also usually readily hydrolysed to ammonium compounds and then nitrified. Hydrolysis of urea is catalysed by the urease enzyme, following the reaction:

$$CO(NH_2)_2 + 2H_2O \rightarrow (NH_4)_2CO_3$$

Besides differences in the chemical form of N, differences exist also in relation to the accompanying nutrients (e.g. P, cations), physical properties, and price per unit dry matter or unit of N. Table I lists the main commercially available N-containing fertilizers.

These N products are applied either alone or in blends combined with other fertilizers, most commonly P and K, according to specific relative nutrient requirements of the crop. Root and tuber crops such as cassava and plantain, for instance, require much more K relative to N than does maize [18]. Di-ammonium phosphate (DAP) is a good fertilizer for grain legumes, as it contains more P than N while the latter may serve as starter N. Besides its grade or specific nutrient content, the physical quality of a fertilizer is determined by its particle size range, its hardness/density, its resistance to moisture and physical damage, and its freedom from

caking [19]. For transport and storage purposes, the specific weight/density is important. Urea has a greater volume per unit of weight than most other fertilizers, making it more bulky to transport and store, which may counteract advantages related to its higher N content.

TABLE I. VARIOUS FORMS OF FERTILIZER N AND SOME SELECTED PROPERTIES [16, 17]

Fertilizer type[a]	N content (%)	Form of N	Other nutrients
Ammonium chloride	25/27	NH_4^+	None
Ammonium nitrate (AN)	33.5/34.5	NH_4^+ and NO_3^-	None
Mono-ammonium phosphate (MAP)	11	NH_4^+	P
Di-ammonium phosphate (DAP)	16/21	NH_4^+	P
Ammonium sulphate (AS)	20.5/21	NH_4^+	S
Ammonium sulphate nitrate	26	NH_4^+ and NO_3^-	S
Anhydrous ammonia	82	NH_3	None
Calcium ammonium nitrate (CAN)	20.5/28	NH_4^+ and NO_3^-	Ca
Calcium cyanamide	20/21	$-NH_2$	Ca
Calcium nitrate	15.5	NO_3^-	Ca
Potassium nitrate	13	NO_3^-	K
Sodium nitrate	16	NO_3^-	None
Urea	45/46	$-NH_2$	None

[a] Compound and complex fertilizers are not listed.

In the developing world, the most commonly used N fertilizers are urea, ammonium sulphate (AS), ammonium nitrate (AN) and calcium ammonium nitrate (CAN). The near-complete replacement of AS by urea is noteworthy (Fig. 1), likely caused by the strongly acidifying properties of AS (see below) and cheaper production of urea after the discovery of large amounts of natural gas in some developing countries.

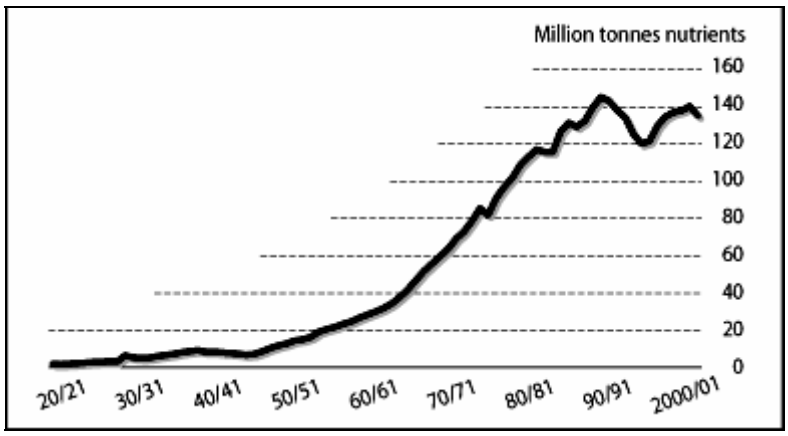

FIG. 1. World fertilizer consumption, 1920/1921 to 2000/2001 [16].

While the number of formulations of N fertilizers is potentially unlimited, the reality in developing countries is often that only a few are available, resulting in the use of inappropriate nutrient combinations and unbalanced fertilization, particularly of food crops. In the Republic of Benin, for instance, cotton fertilizer (14% N, 23% P, 14% K, 5% S, 1% B) — virtually the only compound fertilizer available — does not fit the specific nutrient requirements of maize [18]. The choice of fertilizer type depends on the targeted crop, local availability/cost and the soil/climate. For soils with a low buffering capacity, it would be unwise to use AS as a major source of N due to its soil acidifying potential (see below), while in humid environments it is better to avoid nitrate based fertilizers as these may be leached too quickly. As the range of N fertilizers available is often limited, little flexibility in determining the type of fertilizer to use should be expected. Mughogho et al. [20] generally observed minimal differences in recovery of N from urea and CAN by maize in sub-humid and humid West Africa and by millet in semi-arid West Africa. Finally, when purchasing N fertilizer from stock keepers, it is important to verify its quality, especially if it has been re-bagged in smaller quantities.

2.1.1.1.1. Fertilizer nitrogen consumption

Before the 1950s, consumption of N+P+K fertilizer was rather low [16]. Since that decade, it has increased expontentially, as has the world's population from 2.5 to 6 billion. Both fertilizer N consumption (total amount) and its fraction of the N+P+K total have also increased substantially (Table II). Currently fertilizer N accounts for about 60% of the total global consumption of fertilizer N+P+K (estimated at 134 million tons).

Table III shows the regional distribution of N fertilizer consumption for the period 1998/1999– 2000/2001. It was low for Africa. The Asian region accounted for about 50% of the total consumption, followed by North America, Western Europe, Latin America and the Middle East.

TABLE II. GLOBAL CONSUMPTION OF FERTILIZER NITROGEN, 1920/1921– 2000/2001 [16]

Year	Nitrogen (million tons)	N as % of total NPK consumption
1920/21	Negligible	—
1930/31	1.30	24
1960/61	10.8	36
1970/71	31.8	46
1980/81	60.8	52
1990/91	77.6	56
1998/99 to 2000/2001 (average)	82.8	60

TABLE III. REGIONAL DISTRIBUTION OF FERTILIZER NITROGEN CONSUMPTION, 1998/1999–2000/2001 [16]

Region	Nitrogen (million tons)	Distribution (% of total)
Western Europe	9.83	12
Central Europe	2.18	2.6
Eastern Europe-Central Asia	2.52	3.0
North America	12.6	15
Latin America	4.86	5.9
Oceania	1.18	1.4
Africa	1.35	1.6
Middle East	4.20	5.1
South and East Asia	44.1	53
World 1998/1999–2000/2001 (average)	82.8	100

Although N fertilizer consumption has increased substantially over the past 20 years even in developing countries, its consumption in sub-Saharan Africa has lagged behind those of Asia and Latin America. An analysis of fertilizer consumption for the countries in the Latin American and Caribbean region was made by Urquiaga and Zapata [21].

Both the world fertilizer N+P+K consumption and its shares in the developed and developing worlds have changed over time. In 1960, the developed world accounted for 88% of total fertilizer consumption (30 million tons), whereas by 2001 the developing world accounted for 63% of total fertilizer consumption (140 million tons). This trend is likely to continue in the future. The trend in fertilizer N shares is similar to that in fertilizer N+P+K consumption [22].

Figure 2 shows the evolution of consumption by fertilizer N product. Urea is by far the dominant formulation, due to the market preference for high-grade fertilizers (lower costs of distribution, storage and handling per unit of nutrient).

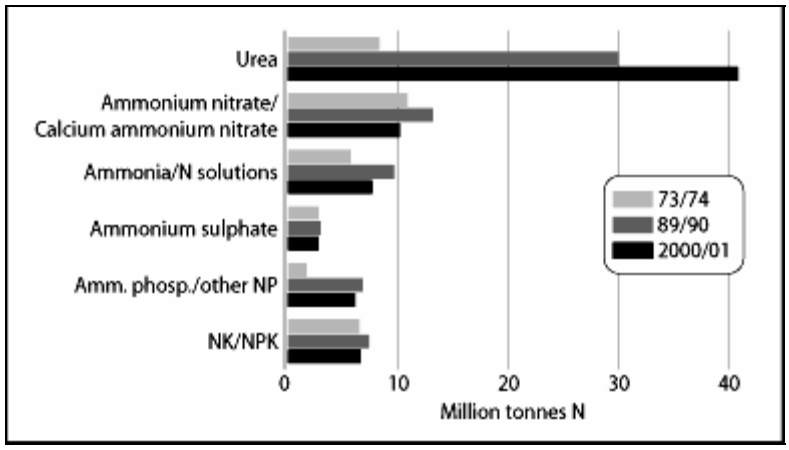

FIG. 2. Evolution of world fertilizer nitrogen consumption (by product) [16].

Since 1992, FAO, IFA and IFDC have organized surveys and compiled statistical data on application rates and fertilizer use by crop for several countries, providing data on changes in cultivated area, food and feed demands and other local factors [22]. The latest editions are available at the FAO/AGL [14] and IFA [17] web sites. From an analysis of global fertilizer application rates for major crops, cereals (wheat, corn, rice, barley, etc.) are the largest users of fertilizers, accounting for about 55% of usage, followed by oilseeds (12%) and pasture/hay (11%). Vegetables, sugar crops, roots/tubers and fibre crops approximately evenly share the remainder fertilizer usage. The crops with highest application rates (above 200 kg nutrient/ha) are banana, sugar beet, citrus, vegetables, potato, oil palm, tobacco, tea and sugar cane [23]. Detailed studies on fertilizer use by crop in Argentina, Cuba, Republic of Korea, Syrian Arab Republic and Uzbekistan were reported recently [15, 17].

2.1.1.1.2. Fertilizer nitrogen production

Regional shares of world fertilizer N production are shown in Table IV. Two countries in the Asian region, China and India, produce over a third, followed by North America and Central and Western Europe, which also account for about a third of the world's production. Production of fertilizer N is much more widespread than that of phosphate or potash. Urea is currently the most commonly produced (in about 60 countries) and utilized fertilizer N.

TABLE IV. REGIONAL SHARES OF WORLD FERTILIZER NITROGEN PRODUCTION (AVERAGE 1998/1999–2000/2001) [16]

Region/country	Annual N production (million tons)	Distribution (% of total)
China	22.0	25
North America	13.9	16
Western and Central Europe	12.4	14
India	10.8	12
EECA	8.63	9.9
Middle East (incl. Egypt and Libya)	6.50	7.5
East Asia	5.20	6.0
Others	7.62	8.7
World 1998/1999–2000/2001 (average)	87.0	100

Figure 3 depicts changes in fertilizer N production that have occurred between the developing and developed worlds over the past 20 years. In 1980, developing countries accounted for 31% of total fertilizer N production (63 million tons), whereas by 2001 their share was 57% (87 million tons).

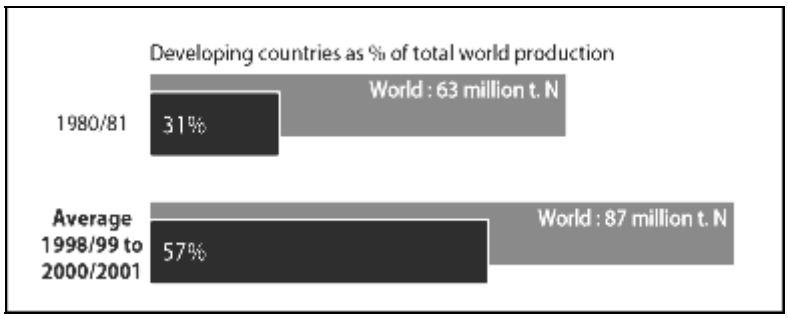

FIG. 3. Fertilizer nitrogen production in developing countries [16].

These trends in production and consumption are likely to continue in the future.

2.1.1.1.3. Forecasting future requirements

In a study titled "Fertilizer Requirements in 2015 and 2030" conducted by USDA, IFA, FAO and IFDC [16], forecasts of total fertilizer requirements were made based on projections of cereal demands for human food and animal feed (Fig. 4) as well as on projections of world fertilizer consumption to 2015 and 2030 (Fig. 5), considering a baseline scenario and a scenario of increased fertilizer efficiency in developed countries, including environmental incentives [15].

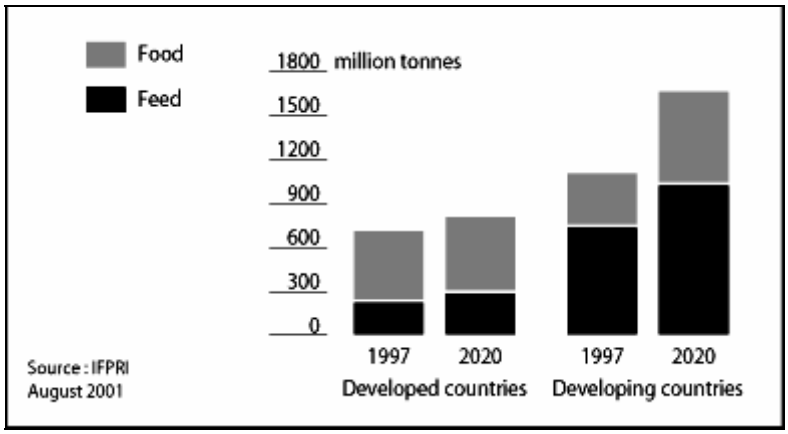

FIG. 4. Projection of world cereal demand for food and feed [16].

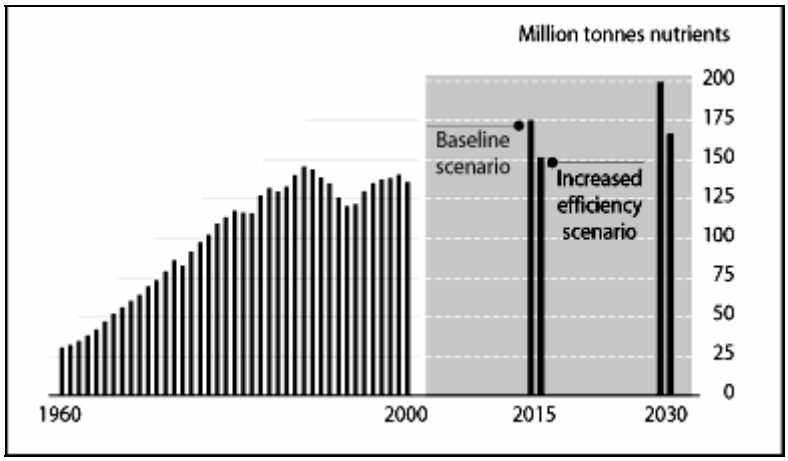

FIG. 5. Forecast of requirements of mineral fertilizers [16].

The annual world fertilizer consumption by 2030 is expected to increase from current levels of 135 million tons to about 199 million tons fertilizer N+P+K (baseline) and 167 millions tons fertilizer N+P+K (increased efficiency), i.e. growth rates of between 0.7 and 1.3% per annum. These forecasted growth rates are lower than those of the past 40 years, which averaged 5.5% annually. Therefore, although this forecast indicates that, to satisfy food and feed demands of the global population, fertilizer consumption will increase, it will do so at a lower growth rate than in the past. Increases in fertilizer use efficiency would help to protect the environment and conserve natural resources [15, 17].

Figure 6 shows the past and future (30 years) fertilizer consumption by region of the world, considering changes in cultivated areas and improvements in fertilizer use efficiency.

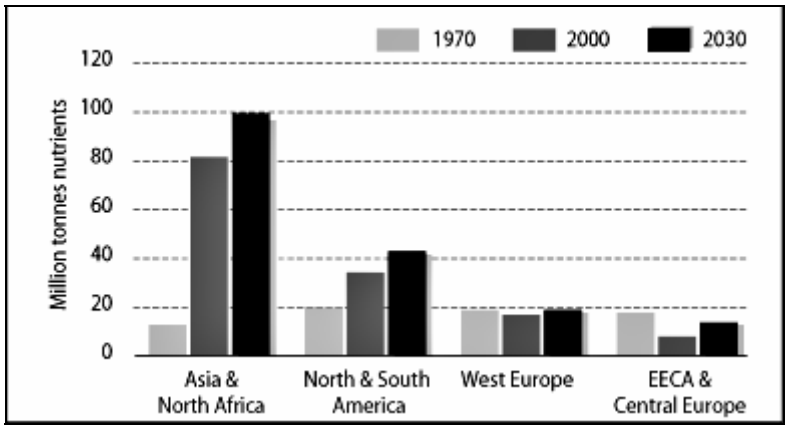

FIG. 6. Projection of fertilizer consumption by region [16].

Major increases in fertilizer consumption are expected in Asia and North Africa and, to some extent, in North and South America. Little change is foreseen for other regions, in particular Europe [15, 17].

2.1.1.2. Need for improving fertilizer use efficiency

Since only a fraction of the applied fertilizer N (on average less than 50%) is taken up by the crop (see Sections 4 and 5), the remainder is subject to loss, representing both economic cost and environmental risk [10, 24, 25].

As huge quantities of fertilizer N are involved in agricultural production systems, the economic losses are enormous. A simple estimate indicates that from the 80 million tons N used in 1996 globally, an average loss of only 20% (with a price of US$0.66 per kg N in urea) represents US$10.6 billion. Other assessments have also been made, taking into account investment costs, energy (fossil fuels) and raw materials required for the production of this amount of fertilizer N [7, 26].

Excessive rates of fertilizer N to obtain maximum levels of crop production are associated with potential environmental, ecological and health risks. In some areas, problems related to the presence of abundant N from anthropogenic sources (including, but not limited to, fertilizers) have been reported. Estimates of the global anthropogenic inputs and a global agricultural output of 23 million tons N per year imply low overall efficiency of N utilization in agriculture for food production [27, 28]. Globally, the total input of N fertilizer is about 80 million tons N/a. The gaseous losses to the atmosphere are estimated at 26–60 million tons

N/a, whereas between 32 and 45 million tons N/a are received by ground- and surface waters through leaching and runoff [29].

In spite of the development of new technologies for improving the efficiency of use — or recovery by crops — of applied fertilizer N over recent decades mainly in the developed world, fertilizer N use efficiency has remained low due to land degradation in many regions of the world, changes in land use (and cropping systems) and the use of inappropriate land management practices in response to socioeconomic and other pressures on agricultural production systems. Fertilizer N not taken up by the crop to which it is applied is very likely to be lost to the environment. This is an important issue for developing sustainable agricultural systems, in view of the need to support further intensification of agriculture to produce enough food for the growing population while preserving the natural resource base. It is now widely accepted that efficiency of fertilizer use should be evaluated not only agronomically (recovery and yield increases, and product quality), but also environmentally and socioeconomically. Recycling and losses must also be considered. The role of fertilizer N should be evaluated within the context of all potential inputs of N to the system as a whole. Due to the multiplicity of cropping systems and influencing factors — including farm management practices — integrated studies must be conducted by multidisciplinary teams both from developed and developing countries, targeting relevant agro-ecological zones and predominant cropping systems [10, 28, 30–33].

As there is still great scope and need for improving fertilizer N use efficiency (FNUE) in cropping systems both in developed and developing countries, this part of the guidelines will deal with relevant aspects of fertilizer N management, with particular reference to the potential use of isotopic tracer technologies in measuring and developing ways of improving fertilizer N use efficiency. The final section includes references and additional recommendations for further reading.

2.1.2. NITROGEN FERTILIZER SOURCES AND INTERACTIONS

Nitrogen fertilizers are judiciously applied to soils with the objective of meeting the N demands of crops, thus ensuring adequate development and yield, provided that other crop growth factors are not limiting. In the soil matrix there is a series of interactions between applied fertilizer N and native soil N. Continuous application of N fertilizers in intensive agriculture also affects soil properties.

Chemical fertilizer N is sometimes applied in conjunction with organic residues in various proportions, resulting in a series of interactions, which are examined in the next section.

2.1.2.1. Interactions between mineral fertilizers and soils

The use of ^{15}N-labelled fertilizers has revealed differences in the uptake of soil N between plants fertilized with N and those unfertilized. Fertilized plants take up more soil N due to the so-called ’priming‘ effect or added nitrogen interaction (ANI). The ANI can be ’real‘ when, for example, fertilizer N increases root volume exploration, or ’apparent‘ when caused by pool substitution or by isotope displacement reactions. Jenkinson et al. [34] produced an excellent review of interactions between fertilizer and soil N, and Hart et al. [35] examined the influence of pool substitution on the interpretation of experiments with ^{15}N.

Scientific evidence does not support a commonly made statement that fertilizers damage the soil. In fact, the opposite is generally found, as fertilizer use increases crop yields and thus increases the amount of organic matter returned to the soil as roots and other residues.

Investigation of long term changes in soil organic matter after forest clearance in Zimbabwe revealed that equilibrium contents of soil carbon (C) under 'high input' commercial agriculture were 32 t C/ha, almost twice as much as the 18 t C/ha on the same soil type under 'low input' smallholder agriculture [36]. The larger amounts of organic C in the soils under commercial agriculture resulted from continuous maize production yielding some 8 t/ha due to large applications of N+P+K fertilizer (approximately 150 kg N/ha, 30 kg P/ha and 30 kg K/ha), compared with continuous maize production of about 1.2 t/ha without fertilizer under smallholder management. Maintenance of organic matter is critical for both structure (in most soils) and soil life, and this Zimbabwean example supports the conclusion that mineral fertilizers will improve soil structure and life where they lead to increases in the soil organic matter stocks. An analysis of several long term trials in West Africa also revealed that organic C contents of plots with fertilizer application were usually comparable to, or slightly higher than, those of plots without external inputs (Table V).

TABLE V. DIFFERENCES IN TOPSOIL ORGANIC CARBON AND pH IN LONG TERM EXPERIMENTS IN THE WEST AFRICAN MOIST SAVANNA ZONE, AS AFFECTED BY APPLICATION OF VARIOUS FORMS OF NITROGEN FERTILIZER (*adapted from Ref. [37]*)

Site (country)	Type of fertilizer[a]	Application rate (kg N/ha)	Duration (years)	Organic C (g/kg)			pH		
				–F	+F	Δ[b]	–F	+F	Δ
Zaria (Nigeria)	AS	24	15	3.1	3.4	+0.3	6.0	5.4	–0.6
Ife (Nigeria)	AS	134	7	8.0	8.5	+0.5	6.3	5.2	–1.1
Many sites[c] (Ghana)	AS	101–330	4–7	NA[d]	NA	NA	NA	NA	(–0.6) – (-0.2)
Ibadan (Nigeria)	AS	150	5	8.7	10.5	+1.8	5.8	4.5	–1.3
Ife (Nigeria)	AS	69	14	5.7	3.5	–2.2	4.4	3.6	–0.8
Bouaké (Côte d'Ivoire)	Urea	160–200	20	13.5	8.3	–5.2	6.0	5.5	–0.5
Ibadan (Nigeria)	Urea	150	5	8.7	9.0	+0.3	5.8	4.9	–0.9
Ibadan (Nigeria)	Urea	60	14	5.9	5.8	–0.1	5.7	5.4	–0.3

Site (country)	Type of fertilizer[a]	Application rate (kg N/ha)	Duration (years)	Organic C (g/kg)			pH		
				–F	+F	Δ[b]	–F	+F	Δ
Ife (Nigeria)	Urea	69	14		8.0	5.7	4.4	3.5	–0.9
Ibadan (Nigeria)	CAN[a]	150	5	8.7	10.4	+1.7	5.8	5.0	–0.8
Mokwa (Nigeria)	CAN	31–188	12	3.1	3.3	+0.2	5.0	5.0	0.0
Ife (Nigeria)	CAN	69	14	5.7	6.5	+0.8	4.4	3.9	–0.5

[a] AS: ammonium sulphate; CAN: calcium ammonium nitrate.
[b] Difference.
[c] Only a range of differences in absolute values is given for a series of 27 sites.
[d] Data not available.

The most common case where repeated use of mineral fertilizers can cause soil fertility problems is the potential for acidification with ammonium based compounds. Where AS or urea are used repeatedly in soils with poor buffering capacity, acidification will occur, and more so with AS than with urea (Table V). This is due to the release of H^+ ions during nitrification of NH_4^+ to NO_3^-. If due attention is taken to ensure that any pH change is corrected by liming or application of organic resources, then such acidification can be avoided.

2.1.2.2. Interactions between mineral fertilizers and organic residues

Due to low availability and/or high cost of mineral and organic N inputs, current soil fertility management strategies often entail combined applications of these inputs [38]. Combined mineral and organic N inputs have the potential for added benefits as a result of positive interactions between them.

2.1.2.2.1. Definitions and theoretical background

Although the concept of interaction between two plant growth factors is inherently present in Liebig's Law of the Minimum, it has recently received new attention in work dealing with the combined application of fertilizer and organic residues. Besides adding nutrients, organic material also provides C as a substrate for soil organisms and may help to break pest/disease cycles. Two hypotheses can be formulated, based on whether interactions between fertilizer and organic matter are direct or indirect. In the context of fertilizer N, which is susceptible to loss if not taken up by a crop, direct interactions are the result of microbially mediated changes in the availability of the fertilizer N due to the addition of available C. The addition of fertilizer N may also affect the availability of soil derived N, although this is likely to be less important when the bulk soil is C limited. Indirect interactions are the result of general improvement in plant growth and increased demand for nutrients resulting from alleviation of another growth limiting factor through the addition of the organic matter.

For N fertilizer, the Direct Hypothesis may be formulated as: temporary immobilization of applied fertilizer N may improve the synchrony between supply of and demand for N and reduce losses to the environment. Observations made under controlled conditions, showing interactions in decomposition or N mineralization between different organic materials [39] or between organic matter and fertilizer N [40], justify the development of the Direct Hypothesis. The Indirect Hypothesis may be formulated, for plant nutrient X supplied as fertilizer, as: any organic matter related improvement in soil conditions affecting plant growth (except nutrient X) may lead to better plant growth and consequently enhanced efficiency of use of applied nutrient X. This growth limiting factor can be at the plant nutritional, soil physicochemical or soil (micro-)biological level. Most of the beneficial effects of mulch or crop rotation could be classified under the Indirect Hypothesis.

Positive interactions based on the Indirect Hypothesis may be immediate through rapid alleviation of growth limiting conditions after application of organic material (e.g. improvement of the soil moisture status after surface application of a mulch) or delayed through the improvement of the soil organic matter status after continuous application of organic residues and associated better crop growth (e.g. improvement of the soil's buffering capacity).

Mathematically, the interaction effect in terms of crop yields can be calculated as:

$$AB = Y_{comb} - (Y_{fert} - Y_{con}) - (Y_{OM} - Y_{con}) - Y_{con} \quad (1)$$

Where AB signifies added benefits, and Y_{con}, Y_{fert}, Y_{OM} and Y_{comb} are crop yields in the control, in the treatments with sole application of fertilizer and organic matter, and in the treatment receiving both inputs, respectively.

The interaction effect may be positive, negative or zero (additive effects only) (Fig. 7).

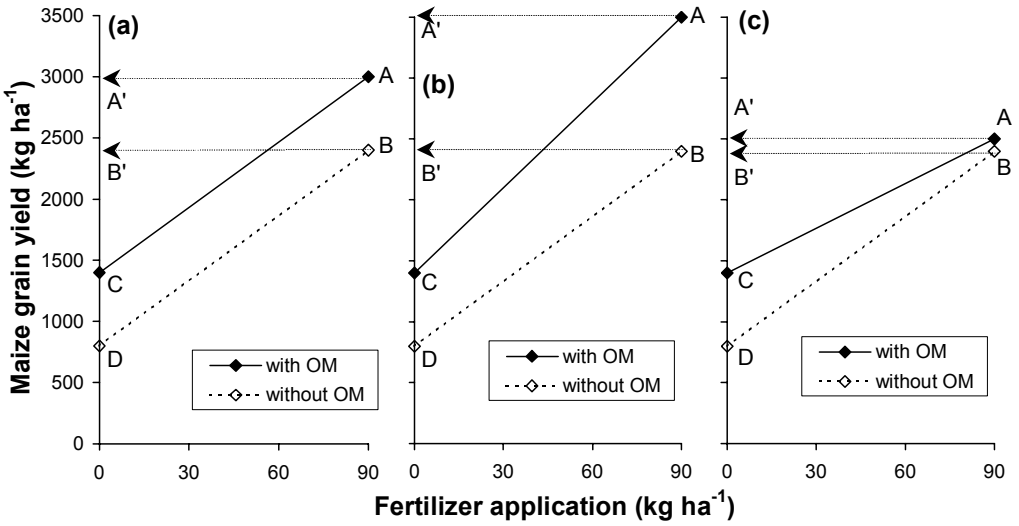

FIG. 7. Theoretical responses of maize grain yield to application of a nutrient as fertilizer in the presence or absence of organic matter. The interaction effect can be calculated as (A' – B') – (C – D) and can be (a) zero, (b) positive or (c) negative. It is assumed that the applied rates of the nutrient are within the linear range of the response curve.

Testing the Direct Hypothesis with ^{15}N labelled fertilizer, Vanlauwe et al. [41] concluded that direct interactions between organic matter and fertilizer N exist not only in the laboratory but also under field conditions. Also demonstrated were the importance of residue quality and mode of incorporation in the extent of these interactions.

In a multilocational trial with external inputs of organic matter, Vanlauwe et al. [32] observed added benefits from the combined treatments at two of four sites, where serious moisture stress occurred during the early phases of grain filling (Table VI). The positive interaction at these two sites was attributed to reduced moisture stress in the 'mixed' treatments compared to sole-urea treatments because of the presence of organic material (surface and subsurface placed), constituting evidence of the occurrence of mechanisms supporting the Indirect Hypothesis.

TABLE VI. CALCULATION OF THE ADDED BENEFITS GENERATED BY MIXING ORGANIC INPUTS WITH UREA AT THREE SITES IN WEST AFRICA

(*adapted from Ref. [32]*)

Site	Y_{mixed}[a]	$Y_{organic}$	Y_{90urea}	Added benefits	Prob. H_0: $AB = 0$[b]
	(Mg/ha)				
Glidji	3.75 (0.20)[c]	2.16 (0.20)	4.19 (0.27)	0.58 (0.26)	NS[d]
Sékou	1.57 (0.06)	0.64 (0.06)	1.52 (0.09)	0.49 (0.08)	$P < 0.001$
Zaria	1.93 (0.12)	0.75 (0.12)	3.14 (0.21)	–0.02 (0.17)	NS

[a] Y_{mixed} is the maize grain yield in the 'mixed' treatment, $Y_{organic}$ that in the 'organic' treatments and Y_{90urea} the yield in the '90 urea-N' treatment. For the '90 urea-N' treatment, the grain yield was derived from the regression analysis relating grain yield with applied urea-N. The data are averaged over the incorporated and surface applied treatments.

[b] Probability that the H_0 hypothesis (added benefit = 0) is true (t test).

[c] Values in parentheses are standard errors of the mean. [d] Not significant.

Although more examples can be found in the literature supporting the Indirect Hypothesis, it is clear that a wide range of mechanisms could lead to improved use efficiency of external inputs. These mechanisms may also be site specific; e.g., improvement in soil moisture conditions would be of little relevance in the humid forest zone. Unravelling these mechanisms, where feasible, as a function of easily quantifiable soil characteristics is a major challenge and needs to be done in order to optimize the efficiency of external inputs. On the other hand, when applying organic resources and mineral fertilizer simultaneously, negative interactions are seldom seen, indicating that even without a clear understanding of the mechanisms underlying positive interactions, the application of organic resources in combination with mineral inputs stands as an appropriate soil fertility management principle.

2.1.3. SOIL TESTING FOR NITROGEN FERTILIZER RECOMMENDATIONS

Nitrogen fertilizer recommendations are based on the amount of mineral N (NH_4^+, NO_3^-) in the soil. These pools, ranging in size from some tenths of kilograms to a few hundred kg/ha, constitute only a small fraction of the total soil N. Several parameters and processes influence the size of the mineral N pools over time. Their (positive and negative) influence is illustrated in Fig. 8. Most of the mineral N is in NO_3^- form because NH_4^+ is quickly nitrified in most arable soils.

Until the 1970s, results of field trials with various N levels over different years were used to identify the optimum N level for a certain crop in a specific region. However, this approach did not take into account the potentially available N in the rooting zone of the crop. Further, because the N requirement is related to the level and quality of production, recommendations change each year, especially with varying weather conditions.

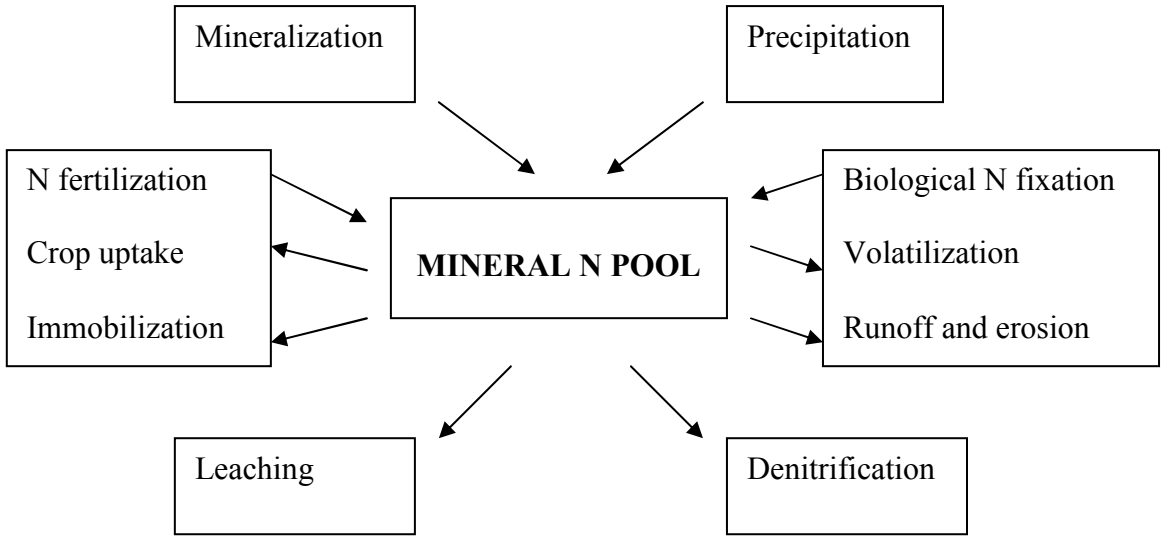

FIG. 8. Processes/factors influencing the soil mineral nitrogen pool.

Optimal N fertilization normally results in crops of good quality. Good timing of the application, e.g. supplementary N at the flowering stage of wheat, can enhance the protein content of the grain. On the other hand, the quality of the harvested product can be negatively influenced by excessive N availability.

Sap purity and sugar extractability from sugar beet and sugar cane, dry matter content and starch content in potato, and nitrate content of leafy vegetables, are examples of traits that can be adversely affected by high levels of available N in the soil. In addition, abundant N can lead to yield reductions, e.g. due to cereal lodging and to higher risks of disease and insect predation. Also, risk of leaching and groundwater contamination is increased with excessive N fertilization. The need for field and season specific N fertilizer recommendations is recognized throughout the world. However, the data and/or the technology to implement a programme to determine optimum N rates on a site specific basis are not always available.

Rapid and accurate determination of mineral N in the soil profile, as well as the availability of plant tissue testing and computer simulation modelling have led to science based N recommendation systems for many crops in various parts of the world [42]. These recommendations can be split roughly into fixed-rate recommendation programmes and variable-rate recommendation programmes. More details can be found in an IFA publication by Hofman and Van Cleemput [9].

2.1.3.1. Fixed nitrogen rates

The simplest fertilizer recommendation specifies a fixed rate for the crop in all situations, regardless of soil type, field characteristics, cultivar, etc. Though easy and without costs for soil or plant analysis, this method is inadequate, as it ignores factors such as mineralizable organic N, residual N from previous applications, rainfall variation, leaching potential for soils of different textures, etc. Nevertheless, it is still in use in many places, especially where possibilities for plant and soil analyses are limited. A refinement of this approach is the UK Agricultural Development and Advisory Service (ADAS) index method [43] utilized in the United Kingdom. On the basis of past management practices and of information of the previous grown crop, fields are attributed an index, ranging from 0 (low amounts of mineral N

(N_{min}) expected) to 2 (high amounts of N_{min} expected), giving an indication of expected N_{min} residues, the exact N_{min} amount being unknown (N_{min} is the amount of mineral N, expressed in kg/ha, in the soil profile to the mean rooting depth of the specific crop at the start of the growing period). The recommended N rate further depends on soil type and organic matter content, as presented in Table VII for winter wheat.

Table VII. NITROGEN INDEX RECOMMENDATION SYSTEM FOR WHEAT (SPRING TOP-DRESSING) [43]

Soil type	Index		
	0	1	2
	(kg N/ha)		
Sandy	175	140	80
Shallow	225	190	130
Deep silty	180	90	0
Clay	190	110	0
Other mineral soil	210	150	70
Organic	120	60	0
Peaty	80	20	0

The lack of precision in such a system is recognized; therefore it is to be used only under conditions where soil sampling is not possible due to the presence of stones, and in situations where N_{min} at the start of the growing period is not likely to fluctuate among fields and years. In all other situations a method that includes soil analysis is recommended [44].

2.1.3.2. Variable nitrogen rates

2.1.3.2.1. N_{min} method sensu stricto

Van der Paauw [45] and others, researching the effects of residual N, were the forerunners for investigations on inorganic N in the soil profile. Later, research in various countries led to N fertilization recommendations based on the linear relationship between the N_{min} in the rooting zone of the crop at the start of the growing period and the optimum N fertilization for the crop.

Table VIII gives an example of an N fertilizer recommendation for potato in the Netherlands as a function of soil type, whereby “a” and “b” represent coefficients of the linear relationships between recommendation and N_{min}. This method, however, is not fully satisfactory. Therefore, other systems that take more factors into account have been introduced.

TABLE VIII. CURRENT NITROGEN FERTILIZER RECOMMENDATIONS IN THE NETHERLANDS (NREC) FOR POTATO [46]

Potato/Soil type	$N_{rec} = a - b \times N_{min}$		Sampling depth for N_{min} (cm)
	A	B	
Ware potato			
Clay and loam soils	285	1.1	0–60
Sandy soils	300	1.8	0–30
Starch potato	275	1.8	0–30
Seed potato	140	0.6	0–60

2.1.3.2.2. Nitrogen index method

The Pedological Service of Belgium also proposed an N index method [47]. Besides the N_{min} amount, other factors, up to a maximum of 18, were included in the N index system. Depending on the history of the field, one or more of these factors could be omitted.

$$N\ index = X_1 + X_2 + X_3 + \ldots. + X_{16} + X_{17} + X_{18} \quad (2)$$

Where X_n represents the various factors.

These factors can be grouped as follows [48]:

— N_{min} (X_1) is the mineral N in the soil profile to the mean rooting depth of the crop at the beginning of the growing period;
— Mineralization (X_2–X_9) are the factors responsible for the N release from soil organic matter and various types of incorporated material: green manure, crop residues, animal manure, compost, etc.;
— Negative factors (X_{10}–X_{18}) are those that have negative effects on N availability: compaction, less than optimum pH, possible N leaching, etc.

The optimum N fertilization recommendation is calculated as follows:

$$N\ recommended = a - b \times N\ index \quad (3)$$

Where a and b depend on the cultivar and the destination of the harvested products.

The relationship between the N index and the optimum N fertilizer rate results in a more precise N fertilizer recommendation.

2.1.3.2.3. Nitrogen balance sheet method

The N balance sheet method was developed in France and in the United States of America [49, 50] and is, with minor adjustments, used also in Belgium and the Netherlands [51, 52]. The theoretically recommended N fertilizer rate is based on the balance between, on the one hand, the N need of the crop plus the amount of residual N_{min} in the soil profile at harvest and, on the other hand, the amount of N_{min} present before planting plus the N mineralization. The residual N_{min} in the soil profile at harvest to the mean rooting depth is the amount of mineral N that remains in the rooting zone after optimum N fertilization. The practical N fertilization recommendation can further be adjusted according to expected losses. These potential losses are estimated at between 5 and 20%, depending mostly on soil texture.

This method has been applied also in China, with the following approach (all parameters are expressed in kg N/ha):

$$W_{input} = W_{output} - \Delta W - (W_n - W_{n+m}) \quad (4)$$

Where W_{input} is the N requirement, W_{output} is the N requirement of the target yield, ΔW is (N mineralized + subsoil mineral N + dry deposition N + wet deposition N) – volatilized N, W_n is available N before planting, and W_{n+m} is available N after harvest.

This method requires significant soil specific data, but does provide a means for making field and season specific recommendations (J. Jin, Chinese Academy of Agricultural Sciences, personal communication, 2004).

The fixedrate and N_{min} methods above do not take into account, or only estimate in the case of the N index and N balance sheet methods, the amount of N that will be mineralized from soil organic matter during crop growth. To better cope with post-planting mineralization, other methods include the determination of whether or not to make an additional N application during the growing season. For example, the pre-sidedress soil nitrate test (PSNT) developed by Magdoff et al. [53] has been widely utilized to estimate the need for supplement N fertilizers in fields planted with maize where large amounts of manure have been applied. This test prevents overapplication of N and provides assurance that adequate N is available to the crop from the organic source, and includes the capacity for top dressing the growing crop. Other reasons for a top dressing are possible improvement in fertilizer N use efficiency, possible yield increase, improvement of quality and decrease in potential adverse environmental impacts. Better N management usually results from split-N-application programmes because it is difficult to predict achievable yields and N losses at the start of the growing season.

2.1.3.2.4. Simulation models

Simulation models make it possible to calculate, on a daily basis, availability of N to a crop, N uptake and crop growth, using average or actual weather data and soil, crop and field parameters as inputs. Simulation models can thus be used to estimate the fertilizer N requirements of a crop at any time during the growing season. Also, environmental side-effects of N fertilizer application can be estimated. These models may be simplified by keeping the number of parameters and the amount of input data to a minimum, depending on the soil, crop and climatic conditions [44]. The main disadvantages of (simplified) models are that they require data that are not always readily available, and that extrapolation is difficult as the models are mostly developed for specific soil and climatic conditions. However, such models can form the basis for determining research needs associated with improving recommendations in areas in which more fertilizer N is being used, as well as for determining the environmental factors (mainly rainfall) that influence optimum N rates from season to season [54].

2.1.3.2.5. Plant analyses (petiole sap analysis, chlorophyll meter readings)

Plant analyses may be used to check the N status of a crop during growth. The idea behind plant analysis is that the crop itself is the best indicator of the supply of N by the soil as well as of the crop's N demand and its ability to absorb the N available in the soil. When the N status appears to be inadequate, additional fertilizer N can be applied. Plant analysis methods

have the advantage that a second N fertilization can be delayed and that the mineral N supply from soil organic matter can at least partly be introduced into the recommendation system. However, the 'translation' of obtained values into amounts of fertilizer N to be applied to compensate for the N deficiency has been, until now, difficult, and optimal timing for a second N fertilizer application is not easy to define.

2.1.3.2.6. Site specific and real time nitrogen management

During the mid-1990s, N omission plots were used to develop a site specific approach to N fertilizer management in rice in Asia [55]. The system involves determination of the N fertilizer need as the difference between the supply of N from indigenous sources (measured with the N omission plots) and the demand of the rice crop for N, as estimated from the total N required by the crop, to achieve a target yield for average climatic conditions. A calibrated leaf colour chart is used to estimate crop N demand through the growing season, and applications are made at predetermined critical growth stages.

The 'real-time' N management approach to determining N needs in rice production in Asia utilizes leaf colour measurements at 7–10 day intervals from 15 to 20 days after planting to flowering [55]. Nitrogen fertilizer is applied whenever the colour values fall below critical thresholds. Preliminary evaluation indicates significant improvement in fertilizer use efficiency in these highly fertilized, irrigated rice production systems. A key component to both the site specific and real time management approaches is that other elements such as P, K and S must be above yield-limiting levels in order for N fertilizer to be used efficiently.

In the United States of America, research groups in Oklahoma and Nebraska have used optical sensors to estimate winter wheat N needs. Sensors measure the normalized difference vegetative index (NDVI) computed from red and near infrared reflectance values. These data are coupled with temporal estimates of N responsiveness and spatial variability in NDVI readings in 0.4 m^2 areas of the field [56]. That research showed increases in N use efficiency of 15% for winter wheat. The principles supporting this technology should apply to estimating N fertilizer requirements for other crops.

2.1.3.3. Nitrogen recommendations in developing countries

Under tropical and subtropical climates, mineralization of soil organic matter is rapid as a result of prevailing high temperatures. Moreover, crop residues are often removed from the field for use as animal feed, etc., or are burned to facilitate land preparation. Although efforts have been made to enhance fertilizer use, it is still marginal, and nutrient balances are often negative. There is substantial variability in fertilizer N use between regions, villages and even fields. Current rates of fertilizer application are often sharply below recommended levels. The many reasons behind low fertilizer use include cost, availability, lack of knowledge of appropriate and efficient use, and often low and/or unstable produce prices, limiting farmers' interest in fertilizer use [57]. Over-attention to organic N might also have resulted in negative opinions on inorganic N. Although organic inputs have an important role, they cannot supply sufficient N for acceptable crop production levels. Integrated soil fertility management (ISFM) advocates the combined use of organic resources and N fertilizers, thereby exploiting the potential for synergy [41].

As for rates of N fertilizer to apply, in many countries standard or 'blanket' recommendations exist, with rules uniformly applied for whole agroecological regions or, in some cases, across the country as a whole. Small scale farmers, however, hardly ever implement such

recommendations. Although discussion of the reasons behind this discrepancy is beyond the scope of this chapter, this clearly indicates the need for flexible guidelines and site specific recommendations, as marked differences are often observed in soil fertility status between fields within a farm belonging to a single household. This variation is caused by inherent soil properties, partly driven by their position in the landscape or "soilscapes" [58], and by farmer induced management effects in different fields.

Carsky et al. [59] have reported a clear positive relationship between soil organic C content and unfertilized maize yields for a number of sites in northern Nigeria. An interesting research issue is whether the returns from N fertilizer application are higher on soils of high fertility status, such as in fields near the homestead, compared with soils of lower fertility status. Soil organic matter content is usually positively correlated with specific soil properties or processes fostering crop growth, such as cation exchange capacity, rainfall infiltration or soil structure. In plots where any of the above factors constrain crop growth, a higher organic matter content may enhance that growth and thus increase demand for N and, consequently, increase the fertilizer N use efficiency. On the other hand, organic matter also releases available N that may be better synchronized with the N needs of the plant than fertilizer N, and consequently a larger organic matter pool may result in lower fertilizer use efficiency. In a trial in western Kenya, relative responses to N fertilizer were stronger on fields of lower soil fertility status (Fig. 9), providing evidence for the second line of thought.

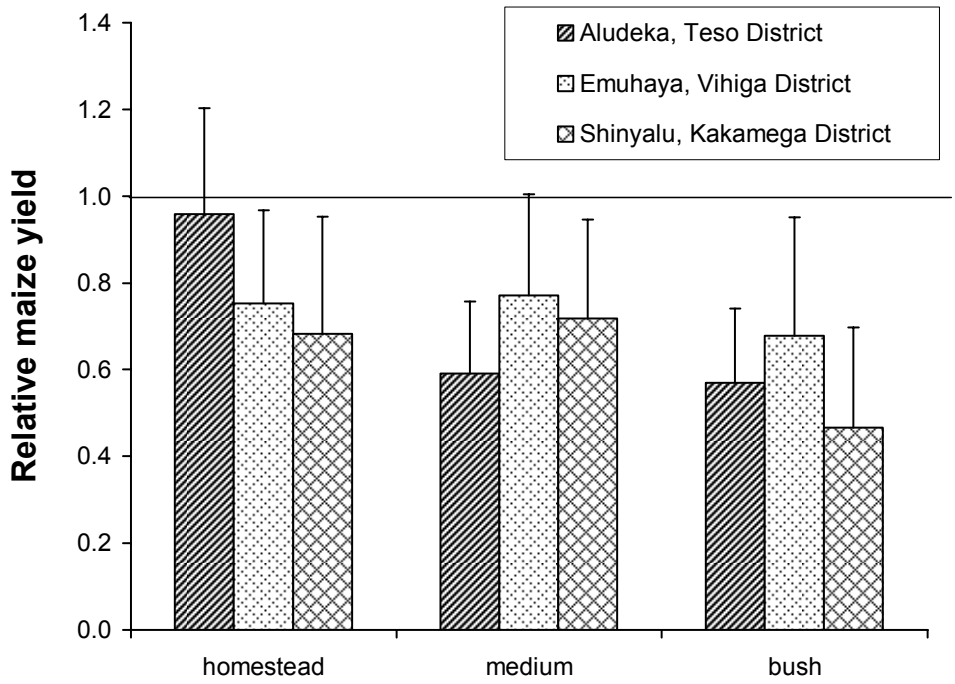

FIG. 9. Relative maize yields (yield in the treatment without phosphorus and potassium applied over yield in the treatment with nitrogen, phosphorus and potassium applied) for fields close to the homestead, at mid-distance, and remote, in Teso, Vihiga and Kakamega Districts, western Kenya. Data are average values of six limiting nutrient strips, and error bars are standard deviations.

Various ways exist to determine crop N requirements for specific fields or areas. Qualitative approaches consider deficiency symptoms in growing plants. Nitrogen deficiency is commonly expressed as follows: (i) stunting and poor plant vigour; (ii) loss of green colour, yellow discoloration of leaves from the tip downward, with older leaves turning brown; or

(iii) lower leaves senesce prematurely while the top of the plant remains green (which can be mistaken for lack of moisture). It is noteworthy that certain symptoms are indicative of more than one nutrient deficiency; symptoms of sulphur (S) deficiency resemble those of N deficiency. Total N analysis of specific plant parts, e.g. the ear-leaf, can assist in determining whether N is lacking. Martin-Prével et al. [60] proposed the following interpretation of ear-leaf N analysis: deficient if %N < 2.45%; low if 2.45% < %N < 2.75%; appropriate if 2.75% < %N < 3.50%; high if 3.50% < %N < 3.75%; and excessive if %N > 3.75%.

Quantitative approaches to assess an optimal N rate are usually based on fertilizer response trials in the area of interest. In contrast with other plant nutrients, practical tests for availability of N are not available, although the pre-season mineral N content of the soil profile has been found to be a good indicator of crop yield in the absence of N fertilizer. Rates of application are then a function of:

— N uptake of the crop to give a desired yield level,
— the N supplied by the soil, and
— recovery of the applied fertilizer N or N use efficiency.

The N supplied by the soil can be estimated by total-N uptake in control plots devoid of fertilizer or in no-N control plots of an N fertilizer response trial. The typical N response curve is not a straight line but reaches a plateau or even declines at a specific N level; it is important to have at least the application rates beside the no-input control in such trials. This allows fitting the response to a non-linear curve.

Alternatively, one could also target replenishing the N removed as harvested products. Such an approach, however, does not take into account current stocks of available N and, depending on the supply of soil N, the applied fertilizer N may be used with varying levels of efficiency. Moreover, if a nutrient balance study indicates a deficit (i.e. an overall removal of the nutrient of interest), then simply supplying that amount of nutrient in the form of mineral fertilizer will not lead to a balanced nutrient budget. Particularly in the case of N, applied fertilizer is subject to loss, particularly through leaching.

An alternative approach is the microdose method, which advocates small applications of fertilizer (e.g. 5 kg N/ha), to be placed near the planting hole. Due to the often observed acute shortages of soil N, responses to such minimal applications of N fertilizer are often substantial, although, in the long term, larger application rates are required where removal of N is greater than the small amount applied.

Guidelines should also be related to likely production, considering that variations in climate — particularly rainfall — determine the potential yield in any given season. Piha [61] developed guidelines for fertilizer applications to maize, in environments where rainfall is unreliable, that increased agronomic and economic efficiencies significantly. The basic approach is to apply less mobile nutrients (P, K, S) at or soon after planting with small quantities of N, then to apply the majority of the N as top-dressing when plant demand is maximal.

Greater attention is needed to such flexible approaches to nutrient management for any given environment in relation to the yields that can be expected if nutrient limitations are removed.

Recommendations should be based on local soil quality indicator schemes rather than on formal soil analysis, as the latter will likely not be accessible to small scale farmers. Such local soil quality indicator schemes provide guides for the farmer’s determination of the

fertility status of a specific plot within his farm. Farmers themselves have ways to classify their soil fertility status based on local soil quality indicators [62]; obtaining insight into this knowledge and linking it with formal assessments of soil fertility status fosters discussion with farmers on soil related problems and solutions. These exchanges can form the basis for making site specific recommendations, as formal soil analysis will certainly remain beyond the reach of many small scale farmers in the developing world.

2.2. MEASUREMENT OF FERTILIZER N USE EFFICIENCY

2.2.1. Definitions

The application of fertilizer N and the efficiency of its use by a crop require the consideration of several distinct criteria [25, 63]. Basic definitions related to N and N use efficiency are provided below.

2.2.1.1. Agronomic efficiency

The agronomic efficiency (AEN) is the amount of harvestable product, i.e. kg of cereal grain, potato tubers, tomato fruit, etc., per kg of applied nutrient (N). Most fertilizer studies focus on this parameter. When determined at various levels of application, the values are called fertilizer–response ratios and are used to evaluate crop response to fertilizer application and the profitability of fertilizer use. This, the classical method for evaluating fertilizer use, is defined by the following equation:

$$\text{AEN (kg grain/kg N)} = \frac{Y_N - Y_0}{F_N} \quad (5)$$

Where Y_N and Y_0 are crop yields (kg/ha) at a certain level of fertilizer application (F (kg/ha)) and in the control treatment, respectively.

For cereals, AEN is often in the range of 10–25 kg grain per kg N applied; it can exceed 30 kg per kg N under optimal management.

2.2.1.2. Uptake efficiency

The N uptake efficiency (UEN) is the total amount of N absorbed (including that present in the roots, often disregarded) per kg of applied N. This ecophysiological parameter, also referred to as recovery efficiency (REN), is defined by the equation:

$$\text{UEN (kg N/kg N)} = \frac{U_N - U_0}{F_N} \quad (6)$$

Where U_N and U_0 are total plant N uptake (kg/ha) in the above ground biomass at a certain level of fertilizer application (F_N (kg/ha)) and in the control, respectively.

Recovery efficiency is often between 0.3 and 0.5 kg per kg, although values of 0.6–0.8 can be achieved. It depends largely on the synchrony between plant N demand and the quantities of N supplied by the fertilizer and by the soil. Consequently, REN is strongly affected by N management methods (see below) as well as by crop management practices (e.g. genotype, tillage, water supply).

2.2.1.3. Plant nitrogen use efficiency

This is the total dry matter or grain yield produced per unit of N absorbed. This physiological parameter, also called physiological efficiency (PEN), is defined as:

$$\text{PEN (kg grain/kg N)} = \frac{Y_N - Y_0}{U_N - U_0} \quad (7)$$

The PEN represents the ability of a plant to transform a given amount of acquired fertilizer N into grain yield and thus depends on genotype characteristics (e.g. harvest index) and environmental and management factors, particularly during reproductive growth. Low PEN usually suggests suboptimal growth conditions, often caused by nutrient deficiencies other than N and/or by drought stress, insect predation and disease.

Plant N use efficiency is related to the other parameters as follows:

$$\text{AEN} = \text{PEN} \times \text{UEN} \quad (8)$$

2.2.1.4. Fertilizer nitrogen use efficiency

Fertilizer N use efficiency (FNUE) is the amount of fertilizer N taken up by the plant per kg of N applied as fertilizer. It is often expressed as a percentage and termed "fertilizer N plant recovery" or "coefficient of utilization."

2.2.2. Parameter relationships

These parameters are interrelated. However, the selection of one or more of them for the evaluation of fertilizer N and overall N use efficiency depends on the objectives of the study. In the present guidelines, the main focus is on the use of isotopic N tracers, in particular the stable isotope ^{15}N, to measure and improve FNUE.

The full concept of FNUE is broader and more complex than the simple recovery of the fertilizer N by a crop, since it involves:

— maximizing plant uptake of the applied fertilizer N;
— minimizing losses of fertilizer N from the soil–plant system; and
— providing/promoting favourable conditions (soil, climate, plant, management, water, etc.) preventing applied N from becoming unavailable to the crop and the cropping system as a whole.

Fertilizer N research has traditionally been concerned with the first aspect, namely obtaining economic yield responses from the crop of interest. Recent research has focused on the second and third aspects, because financial support has been allocated primarily to environmentally oriented projects, in particular in developed countries. However, an integrated approach to all these aspects of fertilizer N management is needed at the cropping system level in selected agro-ecological zones.

Efforts to improve FNUE involve controllable and uncontrollable factors. Figure 10 shows possible sources and estimates of reductions in fertilizer efficiency due to controllable factors.

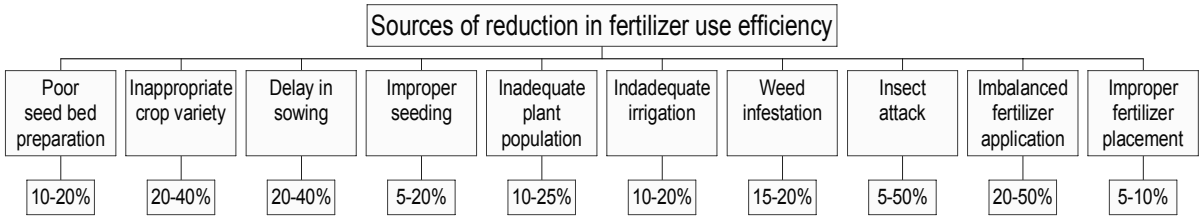

FIG. 10. Factors influencing fertilizer use efficiency [64].

The three main controllable factors that adversely affect fertilizer use efficiency are unbalanced fertilization (20–50% reduction), inappropriate crop variety (20–40%) and untimely sowing (20–40%). The last is especially important in rainfed cropping systems [64]. However, the main uncontrollable factor is climate, which influences both soil and fertilizer N transformations and, consequently, their availability and uptake by plants, and thus crop growth and development. This factor is known as the "year" or "season" effect in multi-year or long term experiments and can be very variable within a location. Prevailing climatic conditions also determine, to a great extent, the pathways of loss of fertilizer N to surface water by runoff, to groundwater by leaching (downward movement within the soil profile) and to the atmosphere as gaseous (NH_3, N_2, N_2O, NO) emissions. The relative significance of climatic conditions and their changes over time need to be taken into account to develop appropriate strategies to improve FNUE.

2.2.3. Measurement methods

Agronomic approaches have traditionally involved the examination of the value of interventions such as fertilizer management practices (timing, placement, fertilizer sources, etc.) and soil/crop management on the uptake of the applied fertilizer N by a crop during a single growing season. Estimates of fertilizer N uptake (FNU) can be made by the non-isotopic difference method as well as by the isotopic method.

2.2.3.1. Difference method

This method is based on the differences in N uptake between fertilized and non-fertilized plants. Plots fertilized with N at several rates and control unfertilized plots are necessary. The nutrient uptake by the crop in the control plots is subtracted from that in the fertilized plots. Recovery data estimated using the difference method are best referred to as 'apparent coefficient of utilization' . Also, the slope of the linear regression relating the plant N content and the rate of applied N can be used.

It is assumed that the nutrient uptake on the control plots measures the amount of nutrient available from the soil, whereas that of the fertilized treatments measures the amount available from soil and fertilizer. This method, furthermore, assumes that all nutrient transformations, i.e. mineralization, immobilization and other processes in the case of nitrogen, are the same both for fertilized and unfertilized soils. Obviously, this is an erroneous assumption, and can account for gross differences between recoveries calculated by non-isotope and isotopic methods [63, 65–68]. It is important to note that the difference method allows the calculation of NUE for a single season, as N in the crop roots and incorporated in the soil organic matter pool is not accounted for. The latter N pools may be quite substantial and contribute to succeeding crops.

2.2.3.2. Isotopic method

The isotopic method is the only direct means of measuring N uptake from applied fertilizer. The recovery data are known as the 'real coefficient of utilization' .

Elemental N has six isotopes, i.e. atoms with the same atomic number (given number of protons, i.e. seven in the case of N) but differing in mass number (number of protons and neutrons in the nucleus, varying from 12 to 17 for N). The isotopes and their main characteristics are listed in Table IX. Of these, ^{14}N and ^{15}N are stable isotopes (defined by their abundance) while the others are radioactive (undergoing disintegration or decay, emitting radiation) with relatively short half-lives, making it difficult to conduct experiments with plants during a growth season.

The stable isotopes, ^{14}N and ^{15}N, have the same number of protons but different numbers of neutrons in the nucleus (Fig. 11).

TABLE IX. ISOTOPES OF NITROGEN

Mass number	Natural abundance (atom %)	Half-life (time)
12	—	0.0126 s
13	—	10.05 min
14 (light)	99.634	—
15 (heavy)	0.366	—
16	—	7.36 s
17	—	4.14 s

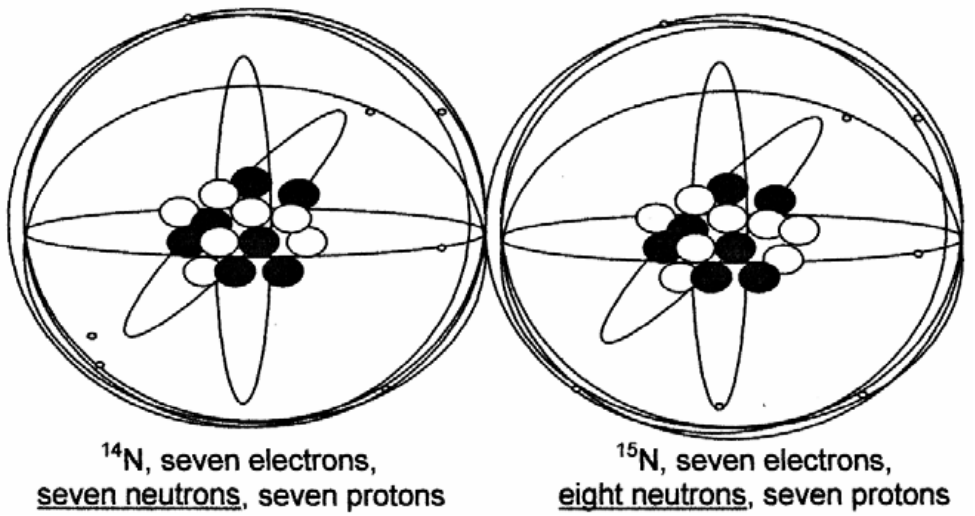

FIG. 11. Stable isotopes of nitrogen.

The more or less constant ^{14}N:^{15}N ratio in the atmosphere and natural substances makes it possible to use N products artificially enriched or depleted in ^{15}N as tracers in ecological systems [69, 70]. Thus, much research work on N in the recent past has used the stable

isotope ^{15}N [69, 71–76]. Since the 1950s and 1960s there has been significant development and use of isotopic tracers in soil and fertilizer N research. Both ^{15}N enriched and ^{15}N depleted materials have been utilized as tracers in a wide range of crops, soils and environments. Pioneering work was mainly carried out in the United States of America, as well as in Canada, the United Kingdom, France, Belgium, Australia and other developed countries. For details, the reader is referred to citations in the References and Further Reading sections.

The Joint FAO/IAEA Programme promoted and coordinated extensive networked research worldwide using fertilizers labelled with the stable isotope ^{15}N to develop improved fertilizer management practices for major food grain crops (see Table II). The results of these projects were reported in a number of IAEA publications [69, 77–81]. Results for grain crops were summarized in an FAO Fertilizer Bulletin [3]. Factors influencing FNUE, such as fertilizer placement, timing and type of fertilizer, and crop management practices such as irrigation, planting density, cropping sequence, etc., identification of N efficient genotypes and competition in mixed agricultural ecosystems have been studied by many research groups [63, 65, 68, 82–86]. A comprehensive review of isotope- ided experiments on fertilizer N management for annual crops carried out in Latin America and the Caribbean region can be found in Ref. [21].

In the past, the major factors that hindered the use of ^{15}N in agricultural investigations were the need for sophisticated and expensive instrumentation to measure the ^{14}N:^{15}N ratio, including sample preparation facilities, and the high cost, and limited availability, of ^{15}N labelled materials [87]. These limitations no longer apply. A whole range of instrumentation is now available for stable Nisotope ratio determinations with increased throughput, precision and accuracy. Due to increased demand, the cost of ^{15}N is now a normal part of the expenses involved in conducting well designed field experiments. As a result, N-isotope tracer technologies are widely used in essentially all developed and in an increasing number of developing countries in agronomic, biological, ecological and related environmental research [73–75].

The following studies related to FNUE have been done using ^{15}N:

— FNUE by annual crops worldwide in a number of environments;
— FNUE by perennial pastures and by fruit and plantation trees;
— FNUE by a crop sequence or the same crop over a number of years;
— Fate of applied fertilizer N or fertilizer N balance/budget in crop and soil, to assess the unaccounted for fraction considered as loss;
— Evaluation of amount and movement of water and associated nitrate leaching;
— Assessment of gaseous N losses (denitrification, ammonia volatilization);
— Interactions with other N sources (soil N, organic sources, biological nitrogen fixation, etc.);
— Fertilizer and soil-N cycling in cropping/farming systems.

The reader is referred to the References and Further Reading sections for detailed and up-to-date information on these topics. IAEA publications listed on the FAO/IAEA web site illustrate the use and applications worldwide of ^{15}N in fertilizer N studies of crops and cropping systems [88].

2.2.4. Nitrogen-15 techniques in fertilizer nitrogen use efficiency studies

Nitrogen-15 techniques, though relatively expensive, usually provide results that have lower variability and are of higher sensitivity, resulting in more precise information in a shorter period of time. In addition, their use and applications require scientific and technical staff with adequate skills and expertise, adequate financial resources and functional laboratory facilities to properly conduct the experiments, perform the isotope measurements and interpret the results. The following sections describe two commonly used approaches of the isotopic method and, in particular, the basic principles for their application.

2.2.4.1. Direct approach

In this case, the fertilizer is labelled with ^{15}N. This requires a commercial source of high quality ^{15}N-labelled fertilizers, i.e. uniform labelling. Thus, direct quantitative measurements of ^{15}N and totalN contents can be made in selected samples, usually plant material. These measurements can also be made for the soil mass (solid phase), soil solution (liquid phase) or soil atmosphere (gaseous phase), depending on the type of study. The following types of FNUE study are possible using the isotope method:

— Fertilizer N management practices (timing, placement, sources);
— Genotypic differences in uptake and use efficiency of N;
— Interactions of fertilizer N with crop/water/soil management variables (irrigation, tillage, cropping system, plant population, soil amendments, plant spacing, etc.);
— Fertilizer N balance/budget determinations (accounted/unaccounted for);
— Direct measurement of labelled fertilizer N losses (leaching/gaseous losses).

In all of these studies, the main goal is to improve FNUE through several approaches. In a first instance, the initial objective is to measure the uptake of the applied fertilizer N and to determine the actual level of FNUE in the system in order to devise ways and means of improvement. Thus, the main goal is to supply N as fertilizer to the plant in the correct amount at the right time and place, matching the crop's needs.

The ^{15}N labelled single-treatment fertility design is a variant of the direct approach to measure FNUE without plant–fertilizer interaction. It has been shown to be a powerful tool to assess the value of fertilizer management practices such as timing, placement and sources [89–91].

The following example illustrates its application to assess the value of ammonium and nitrate as sources of N in a single fertilizer trial of flooded rice. Ammonium nitrate was ^{15}N labelled either in the ammonium or nitrate ion. Table X shows FNUE data obtained with flooded rice in five countries, examining the effects of placement and N source.

TABLE X. PLACEMENT OF NITRATE AND AMMONIUM AS SOURCES OF N TO FLOODED RICE

Country	N source	Surface application (%Ndff)[a]	Incorporated at 5 cm depth (%Ndff)[a]
Republic of Korea	$*NH_4NO_3$[b]	11	14
	NH_4*NO_3	2.2	2.0
Egypt	$*NH_4NO_3$	7.0	11
	NH_4*NO_3	4.7	0.8

Country	N source	Surface application (%Ndff)[a]	Incorporated at 5 cm depth (%Ndff)[a]
Hungary	*NH_4NO_3	7.0	14
	NH_4*NO_3	2.4	3.0
Sri Lanka	*NH_4NO_3	10	20
	NH_4*NO_3	5.9	2.4
Burma	*NH_4NO_3	12	20
	NH_4*NO_3	2.7	2.5
Average	*NH_4NO_3	9.3	16
	NH_4*NO_3	3.6	2.1

[a]Percent N derived from fertilizer..
[b]Asterisk denotes the ^{15}N labelled atom.

All fertilizer N treatments were identical with regard to the total N rate (100 kg N/ha) and the placement methods: either to the surface or incorporated at 5 cm depth. Only the N source marked with an asterisk was ^{15}N labelled, enabling the effect of source to be assessed without plant–fertilizer interaction for each placement method. The average and individual country %Ndff data clearly demonstrated the value of the isotopic method for investigating the optimum placement method for each of the fertilizer N sources. In all five countries, ammonium was the better source of N, and the ammonium-N use efficiency increased from 2.6 (surface application) up to 7.5 times by incorporation at 5 cm depth in comparison to the corresponding nitrate placement treatment. The effects of these sources on grain yield and N accumulation were also studied [77, 92].

The significance of changes in climatic conditions over time needs to be taken into account when FNUE studies are conducted in multilocation trials in selected agro-ecological zones and cropping systems to further undertake fertilizer N budget studies by measuring inputs and outputs (losses) and to develop appropriate strategies to improve FNUE [68, 86, 93–97]. A better approach for a more comprehensive overview of the N cycling and soil organic matter turnover is to implement long term experiments in representative sites; however, this requires more and sustained resources over time [98, 99]. Pieri [100] and Martius et al. [101] have analysed relevant aspects related to the management of soil organic matter in West African savannah and humid regions.

2.2.4.2. Indirect approach

Where it is not possible to isotopically label the N source(s), the isotope dilution method, in which a ^{15}N labelled common source is added as tracer to the soil, is utilized to obtain information on the N supply from the source(s) under study.

The technique requires the inclusion of a ’standard‘ or reference treatment with the only addition of the ^{15}N labelled material, omitting the test unlabelled source. Other experimental treatments include the addition of the same ^{15}N labelled material and the test unlabelled source (at different rates, or several unlabelled sources). The extent of decline in the ^{15}N:^{14}N ratio of the treatment with the unlabelled source with regard to the standard treatment indicates the N availability from the source to the plant. It has been extensively used to measure biological N_2 fixation by field grown legume crops [102, 103], woody legumes,

woody perennials [104, 105], endophytic N_2 fixation in cereals and grasses [106–108], the contribution of *Azolla*-N to the rice crop [109, 110], and the N supply from various organic materials such as guano, compost, animal manure and slurry, sewage sludge, green manures, crop residues, and agroindustrial wastes [63, 111, 112].

Some authors have utilized this technique to assess the residual effect of N fertilization (unlabelled N applied to the first crop) on a succeeding crop in a rotation system and to estimate the residual N effect of an N_2-fixing legume on a succeeding cereal [113–115].

Thus, this technique allows the measurement of N supply from natural products and organic materials that are not possible to label without alteration of their properties, and ultimately their quality. Another advantage of this method is that it is usually cheaper, faster and easier to implement than the direct approach. For more details on its application and illustrations, see Refs [116–118].

This technique can also be used to measure the N supply from inorganic N fertilizers that cannot be easily labelled or for which labelling would be very expensive, such as controlled-release formulations or urea of unusual particle size [68].

2.2.4.3. Conclusions

Experiments with ^{15}N labelled fertilizers, using either the direct or the indirect approach, provide precise and quantitative data on the efficiency of use, residual effect, movement and transformation of fertilizer N. The use of the isotopic method provides a direct and quick means to obtain the needed information, resulting in higher economic return. This information is valuable both for the design of better fertilizer N strategies and for the provision of sound recommendations for the application of fertilizer N.

Although extensive field research has been made on FNUE and a wealth of information has been collected, more work is needed to adopt an integrated approach to the management of crop, soil, water and N sources (fertilizer N, N_2 fixation, organic residues). More research is needed to gather long term data on N (and carbon) accumulation and cycling processes occurring at the cropping system level that are essential for proper assessment of the value of interventions designed to improve the overall N use efficiency of agro-ecosystems, with the ultimate goal of enhancing sustainable intensification of agricultural production.

2.2.5. Field experimentation techniques for the nitrogen-15 method

Isotope aided studies involve the use of labelled materials as tracers for quantitative determination of the fate of specific nutrient elements in a specific component or the whole soil–plant system. In the case of N, the isotope ^{15}N can be traced in each or all of the components of the soil–plant system, such as plants (and their parts), soil mass (soil phase), soil solution (liquid phase) or soil gas (gaseous phase), depending on the objectives of the study.

2.2.5.1. Introduction

The planning and implementation of isotope aided studies require a different approach from that followed in the design of conventional fertilizer trials, due to the cost and often limited supply of labelled materials, collection and preparation of samples, the use of specialized analytical techniques and measuring equipment, and the need for skilful staff trained in the

use of isotope techniques in the field, greenhouse and laboratory. Reviews of these topics are available [90, 91, 114].

At the planning stage it is advisable that the research team establish connections with appropriate research institutions, extensions and farmers' associations/communities to ensure broad input on the definition of the problems to be solved, gathering of background information, implementation of the studies and dissemination of the results to beneficiaries and end users. Experience gained in developing countries through FAO/IAEA Technical Cooperation Projects has demonstrated that the best results are obtained when collaborative agreements are established between specialized groups of national agricultural institutes/universities and nuclear experts from atomic energy institutions. Similarly, in FAO/IAEA Coordinated Research Projects, such collaboration and networking is established between agricultural groups of developing and industrialized countries to promote the sharing of knowledge and exchange of experience enhancing synergies to develop new technologies [33].

Since the isotopic method is normally complementary to conventional or classical methods in agricultural investigations, the research team should ideally consist of scientists not only trained in the use of the method but also skilled and experienced in field experimentation.

When conducting isotope aided fertilizer studies, first we must consider the current status of implementation of a national, regional or local fertilizer programme. Figure 12 is a flow diagram showing the overall sequence of steps required to generate fertilizer recommendations in a country, up to their adoption by the farmer.

The process of generating this information is laborious and time consuming, and requires proper infrastructure involving participation and coordination of public and private organizations, sufficient resources (human, physical and financial), in particular adequate socioeconomic conditions, policies, etc. The specific set-up of a fertilizer programme depends on particular conditions prevailing in the country of interest [15, 89].

Although it is known that isotopic techniques are a powerful tool in agricultural research, in deciding to use them to full advantage, one must consider if the following criteria are met:

— the isotopic method is the only way to solve a particular question or to obtain a particular piece of information, and
— if other methods are available, the isotopic method is a quick and cost effective means to obtain the needed information.

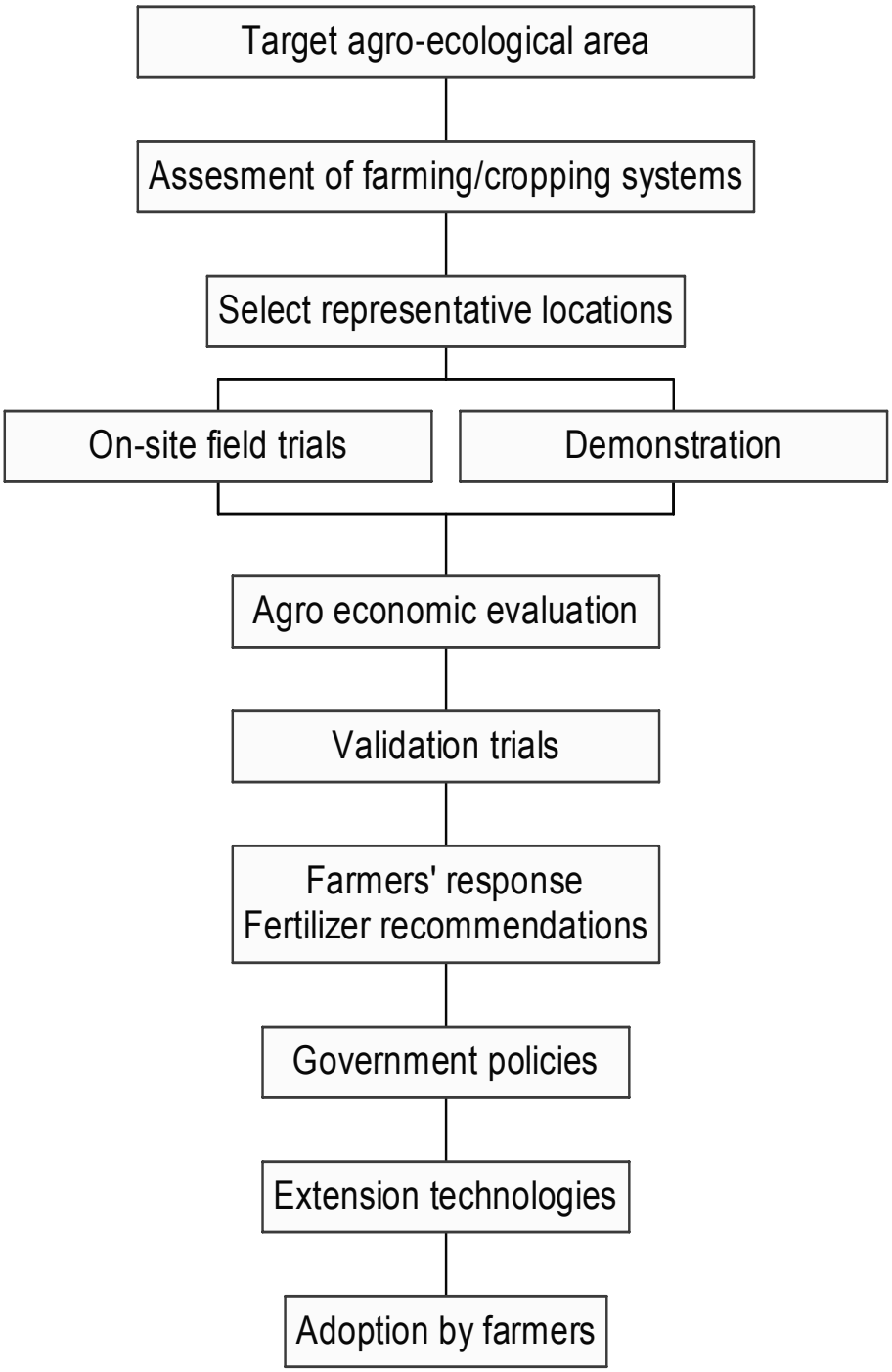

FIG. 12. Implementation of a national/regional fertilizer programme.

In the context of fertilizer studies, it is essential to determine first when and where the isotope method will be applied during the experimentation phase. This is shown in the diagram in Fig. 13, where the isotope method is utilized mainly in phase II to refine and improve existing fertilizer management practices. It is evident that the method must be used only when it is advantageous and cost effective under local conditions.

Therefore, correct application of the ^{15}N techniques is absolutely necessary to obtain high quality data and the valuable information desired. This, in turn, demands that adequate field experimentation techniques (field experiment layout, plot design, application of N labelled products, chemical and isotopic analyses, data calculations, etc.) be utilized. The following

sections deal with field experimentation techniques and provide guidelines on essential aspects of their application.

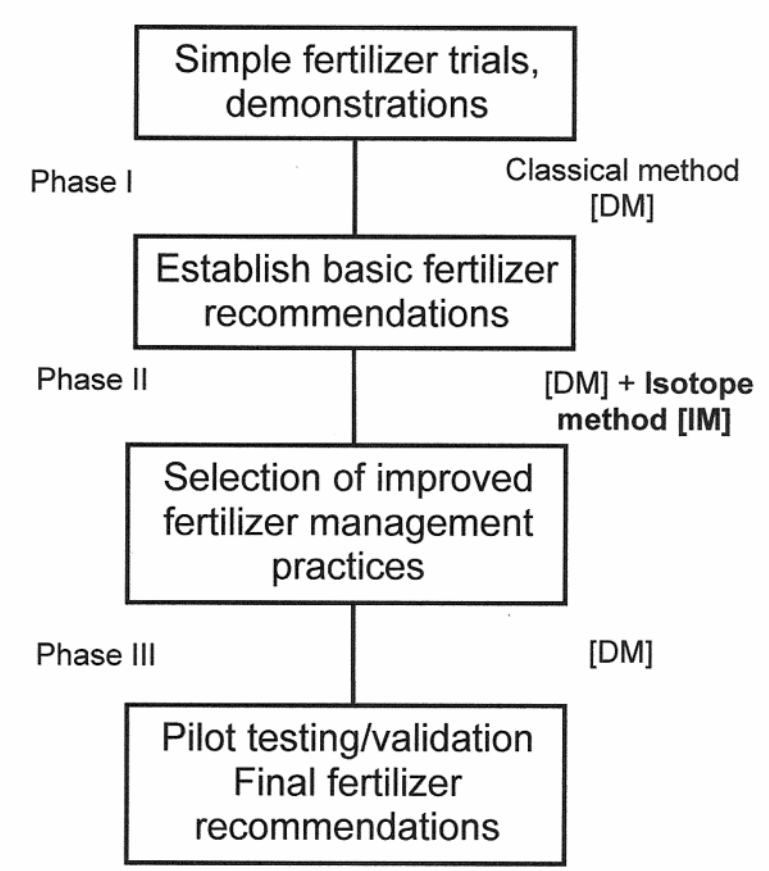

FIG. 13. The use of the isotopic method in fertilizer studies.

2.2.5.2. Experimental guidelines

Detailed planning of an experiment using isotopically labelled fertilizers should include preparation of an experimental guideline, which, after review by the research team, is distributed among all staff. Main points to be taken into account in planning and implementing any isotope aided FNUE experiment are the following:

— Identify the specific fertilizer-N related problem(s) to be studied, define the topic(s) of research and establish priorities of the work to be done. These aspects should be reflected in the title of the study.
— Compile background information (recent and relevant work) on the topic(s) from the scientific literature, databases, reports, etc. Perform a bibliographic search, utilizing key words from the above sources. In particular, define the role of the isotopic techniques in the study. Prepare a list of selected key references.
— Define the objective(s) of the experiment/question(s) to be answered/hypotheses to be tested. A golden rule is to design simple experiments with concrete and well defined objectives.
— Define type and location of the experiment(s): greenhouse, on-station, farmer's field, sequence.

— Establish the experimental treatments and select the appropriate experimental design, replicates, statistical analysis (software), tests for comparisons of means and error estimates. Determine plot layout design.
— Estimate the approximate amount of ^{15}N product(s) and enrichment.
— Define sampling/harvesting times and procedures. Estimate the total number of samples to be analysed.
— Analytical methods, laboratory standards, quality control, data reporting.
— Calculation of data and selection of the evaluation parameters in relation to the objectives of the study.
— Compile all information and develop guidelines for the experiment.
— Assess the resources (physical, human and financial) needed to conduct the experiment, including a budget and sharing among institutions.
— Revise the draft experimental guidelines (working copy) with relevant staff members.
— Distribute the final experimental guidelines among all participating staff.

2.2.5.3. Practical experimental procedures

In addition, the following practical experimental procedures and techniques should be considered for isotope aided field experiments. In principle, the same guidelines are valid for isotopic experiments performed in the greenhouse. For detailed information on these topics, refer to IAEA manuals [90, 91].

2.2.5.3.1. Experimental site

Select a representative location for the problem/topic to be studied and the predominant cropping system in the agro-ecological zone of interest (see Fig. 12). In a regional or national programme, multilocational trials are established to obtain information on FNUE. This is normally done through the conduct of ’on-station‘ and ’on-farm‘ trials, to avoid significant ’yield gaps‘ due to differences in soil fertility and management practices between experimental stations and farmers’ fields, and to facilitate the transfer of the generated technologies to beneficiaries and end users.

The normal approach is to proceed stepwise, starting with detailed on-station experiments followed by on-farm field trials with a simplified experimental design (reduced experimental treatments, with each farmer considered as one replication within a particular location) with farmer participation to facilitate adoption. Sometimes, when time is too short to generate fertilizer recommendations, both can be performed simultaneously; however, this requires more resources, and tighter control and supervision on the part of the local staff.

2.2.5.3.2. Treatments and experimental design

The experimental design should be established in direct relation to the objectives of the study. The number of treatments and replications and the statistical design are a function of each experiment. The final decision on the total number of experimental units should be based both on technical and economic considerations.

Basic principles of statistical analysis and biometrics should be considered in selecting the appropriate experimental design [90]. Past experimental plans of cooperative research projects on FNUE included a core of 4–6 mandatory treatments (common to all investigators participating in the project) and 2–4 additional treatments (as an option of each investigator to address local factors/issues). Randomized block arrangements with 4–6 replications per

treatment were the most commonly used statistical design. Also, the split-plot design has been used in several FNUE experiments. Select appropriate software for statistical analyses (ANOVA) and tests for comparisons of means. A statistician's advice may be useful.

2.2.5.3.3. Plot layout

In isotope aided experiments, two types of plot are required: yield and isotope plots. The plot layout depends on the plant species/variety and the cropping system [68, 119].

Isotope plots are the smallest possible area (usually called microplots) to obtain a representative sample for isotope enrichment measurements while reducing the amount of isotope utilized due to its cost. Microplot sizes may vary from about 1 m^2 (pastures, small grain cereals) to about 10 m^2 (widely spaced crops); they usually contain at least 20 plants. In some crops (pastures) they may contain a higher number of plants, while in others (with wide row spacing) fewer plants, and in some cases just one plant (e.g. a tree), may be involved. Yield plots must be sufficiently large to obtain precise information on yield parameters (total biomass and economic crop yield) and for other additional observations (crop growth measurements, physiological parameters, soil water measurements, plant and soil samplings, etc.) to be made throughout the growth cycle of the crop; their size is usually several (5–10) times the microplot size.

Another consideration is the use of unconfined and confined plots. Both types show advantages and disadvantages. Saffigna [120] and Follett [121] have made detailed reviews on this topic. When using unconfined plots, a consideration of 'border effects' is absolutely necessary. In a microplot, the harvesting/sampling area is the inner part (plants/soil located in the inner central rows), leaving aside the border areas (outer/guard rows at both sides and extreme ends of central rows). The aspects described above are illustrated in the attached diagrams of a main plot comprising both yield and isotope subplots in a fertilizer use experiment (Fig. 14) and an isotope microplot for an experiment on N_2 fixation (Fig. 15).

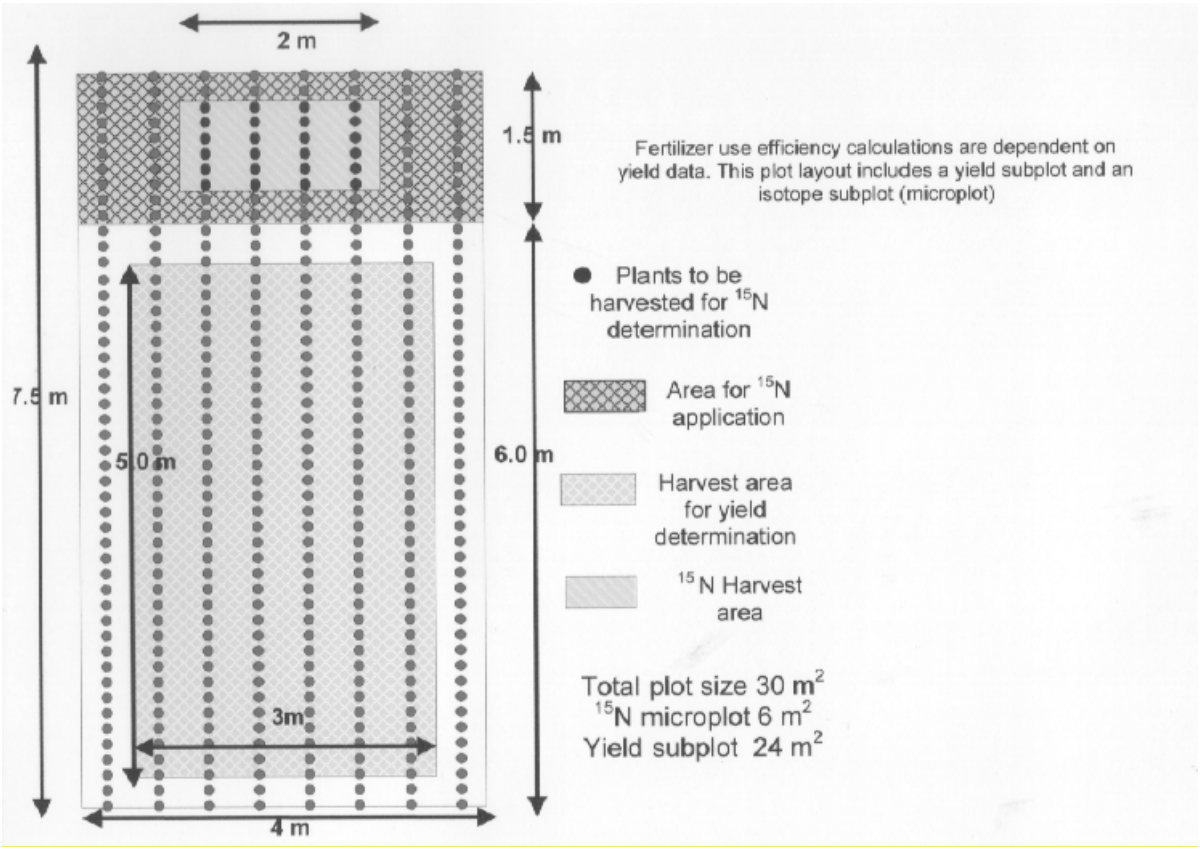

FIG. 14. Main-plot layout for an experiment on fertilizer use efficiency.

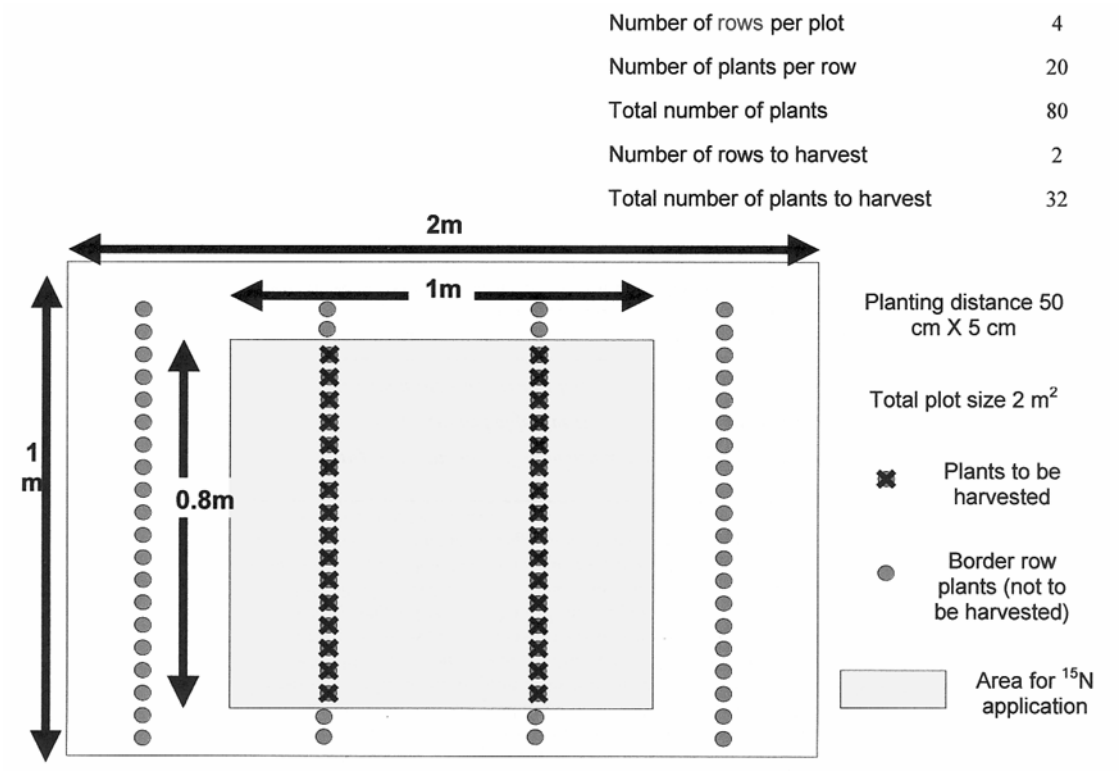

FIG. 15. Plot layout for a nitrogen fixation experiment using nitrogen-15 (soybean).

2.2.5.3.4. Field layout

With regard to field layout, several approaches have been utilized. The experimental layout or the spatial arrangement of the plots (yield and isotope plots) depends to a large extent on the objective of the experiment and on local field conditions (available area, field orientation, slope conditions, etc.). A common approach is to have main plots, subdivided for yield, and isotope subplots with a microplot each located adjacently or within it. The main plots are assigned the experimental treatments and are located in the field according to the design. Another approach is to have the microplots scattered within the inner part of the field, according to the design, and the yield plots distributed randomly in the outer area [68, 119].

Preparation of diagrams illustrating the field and plot layout is key for conducting field observations by a research team and for ensuring efficient establishment, maintenance, sampling and harvesting operations by the field staff [119].

Khaniff et al. [122] confirmed the validity of the assumption of utilizing ^{15}N microplot data for extrapolation to the whole field. Yield, N uptake and variability in yield and isotope-plot data were compared using the t test and double-tailed F test. As no significant differences were found, it was concluded that the results from the ^{15}N microplots could be extrapolated to the field as a whole. An additional advantage is that, due to limited border effects, the same amount of fertilizer could be used to treat a larger area than with the common approach, whereby the microplots are scattered in the field [68].

2.2.5.3.5. Requirements for nitrogen-15 labelled materials

In most FNUE experiments to define the best fertilizer management practices or fertilizer-N balance studies using the isotope method, only one rate of application of fertilizer N is utilized, namely that normally recommended to obtain optimum yield. Where split applications are recommended, that single rate of application, for instance 100 kg N/ha, can be divided into two (50 kg N/ha), three (33 kg N/ha) or four (25 kg N/ha) equal fractions to be applied at selected stages in the growth cycle to study the time course of N uptake (and recovery) by the crop. In other cases, the splitting can also be made in different amounts to best match crop needs, depending on the objectives of the study and the local conditions.

In some experiments where interactions between fertilizer N and other factors are being studied — irrigation, planting density, tillage, residual effects, etc. — different N applications may be utilized, including the recommended rate as intermediate.

When utilizing the direct approach, a control or check treatment (without fertilizer N application) is, in principle, not needed because fertilizer N uptake is measured directly using the ^{15}N labelled fertilizer. However, some authors have included it in order to gather additional information (e.g. comparing difference-method data) and for economic evaluations.

In the indirect approach, the standard or reference treatment (^{15}N labelled but without application of the unlabelled fertilizer-N source) is always needed. This treatment provides baseline information on the established N–isotope ratio under the experimental conditions.

The amount of ^{15}N applied as fertilizer must be sufficient to be detected eventually in the plant samples collected. It depends both on the rate of application and the enrichment (^{15}N% atom excess) of the labelled fertilizer/material used and is determined by several factors such

as the objective of the study, type of crop, duration of the experiment, and primarily the available equipment for measuring the N–isotope ratio.

As a rule of thumb, 1 kg ^{15}N/ha (0.1 g pure $^{15}N/m^2$) or 2 kg ^{15}N/ha (0.2 g pure $^{15}N/m^2$) is needed for FNUE studies with annual crops (plant recovery) if N–isotope ratios are measured by mass or optical emission spectrometry, respectively. As a general guideline, please refer to Table XI.

TABLE XI. FERTILIZER NITROGEN RATES AND NITROGEN-15 ENRICHMENTS COMMONLY USED IN FIELD EXPERIMENTS

Type of study	Rate (kg N/ha)	Analyses by optical emission spectrometry (atom % ^{15}N excess)	Analyses by isotope ratio mass spectrometry (atom % ^{15}N excess)
FNUE studies (annual crops)	33	6	3
	50	4	2
	100	2	1
N_2 fixation studies (grain/pasture/forage legume crops)	5	40	20
	10	20	10
	20	10	5
Fertilizer N studies (crop rotations/fruit trees/plantation crops)	100	20	10
Fertilizer N balance	100	20	10
Soil/plant studies	200	10	5

In fertilizer N split-application studies, the ^{15}N enrichment values of the applied fertilizer N will change according to the application rate. The same applies to biological N_2 fixation studies, with the difference that the applied N rates are always low (to minimize interference with the N_2 fixation process). The lower the N rate, the higher its ^{15}N enrichment has to be, thus resulting in the same amount of ^{15}N applied. In fertilizer-N balance studies (recovery in plant and soil) and rotational/sequential studies over a number of years, about ten times as much as the rates mentioned above are required (10 kg ^{15}N/ha or 20 kg ^{15}N/ha), depending again on the measuring equipment and local conditions.

In special studies — of downward movement of ^{15}N labelled fertilizer, incorporation into soil-N fractions, leaching and runoff losses, ammonia volatilization, etc. — much higher rates (at least 50 kg ^{15}N/ha) are required. However, it should be noted that there is no general recipe, and data published from other experiments are only rough guidelines. In all cases, the exact amount of ^{15}N to be used for any experiments must be tested and defined by the researchers themselves. With experience, the investigators will gain first-hand understanding of the local conditions, to aid in the choice of adequate amounts of ^{15}N needed for their own experiments.

2.2.5.3.6. Calculation of requirements for nitrogen-15 labelled fertilizer

It should be noted that isotopically labelled fertilizers are chemically pure compounds and not simple commercial fertilizers. Thus, calculations must be made following basic N isotope terminology and stoichiometry.

Example 1

Table XII shows the relationship between M (molecular weight or molar mass (g/mol)) and W_N (%N content) for the most common N compounds with ^{15}N natural abundance (a_o = 0.3663 at.% ^{15}N).

TABLE XII. FORMULAS, MOLAR MASS AND PER CENT NITROGEN CONTENT OF CHEMICAL COMPOUNDS COMMONLY USED AS FERTILIZER N

Compound	Formula	Molar mass (g/mol)	%N
Ammonium sulphate	$(NH_4)_2SO_4$	132.14	21.20
Urea	$(NH_2)_2CO$	60.06	46.64
Ammonium chloride	NH_4Cl	53.49	26.19
Ammonium nitrate	NH_4NO_3	80.04	35.00
Sodium nitrate	$NaNO_3$	84.99	16.48
Potassium nitrate	KNO_3	101.11	13.85

Example 2

If the compounds are ^{15}N enriched, they will have different A_N (average atomic weight of N), M and W_N values (Table XIII). This is illustrated by the following example for ammonium sulphate with 10 at.% ^{15}N abundance

TABLE XIII. A_N (AVERAGE ATOMIC WEIGHT OF NITROGEN), MOLAR MASS AND W_N OF AMMONIUM SULPHATE WITH DIFFERENT ATOMIC PER CENT ^{15}N ABUNDANCE

^{15}N abundance (at.%)	A_N (g)	Molar mass (g/mol)	W_N (%)
Natural (0.3663)	14.0036	132.14	21.2
10	14.10	132.33	21.3
50	14.50	133.13	21.8

In the case of ammonium sulphate with 10 at.% ^{15}N abundance:

$$A_N = \frac{\alpha \times 15 + (100 - a) \times 14}{100} = \frac{10 \times 15 + 90 \times 14}{100} = \frac{1410}{100} = 14.0 \text{ g}$$

M = 28.2 + (8.0632 + 32.0640 + 63.9976) = 28.2 + 104.13 = 132.33 g/mol

$$W_N = \frac{28.2}{132.33} \times 100 = 21.3\%$$

From the above, it is clear that the exact amount of ^{15}N present in labelled fertilizer should be calculated.

Example 3

When ordering ^{15}N labelled fertilizer and comparing bid quotations from suppliers, it is important to note whether the ^{15}N enrichment is expressed in ^{15}N abundance or in atom % ^{15}N excess. Table XIV shows the differences in ^{15}N content in 1000 g ammonium sulphate and urea expressed at various ^{15}N enrichments.

TABLE XIV. NITROGEN-15 CONTENT IN UREA AND AMMONIUM SULPHATE AT SEVEN LEVELS OF ENRICHMENT

^{15}N excess (at.%)	In 1000 g ammonium sulphate		In 1000 g Urea	
	g ^{15}N excess	Total g ^{15}N	g ^{15}N excess	Total g ^{15}N
1	2.27	3.10	5.00	6.82
2	4.54	5.37	9.99	11.8
3	6.81	7.64	15.0	16.8
4	9.08	9.91	20.0	21.8
5	11.3	12.2	24.9	26.8
10	22.7	23.5	49.8	51.6
20	45.3	46.1	99.3	101
^{15}N abundance (at.%)				
1	1.44	2.27	3.17	4.99
2	3.71	4.54	8.16	9.98
3	5.98	6.81	13.2	15.0
4	8.25	9.08	18.1	20.0
5	10.5	11.3	23.1	24.9
10	21.8	22.7	48.0	49.8
20	44.9	45.3	97.5	99.3

2.2.5.3.7. Calculations of nitrogen-15 labelled fertilizer requirements

Following the guidelines above, the total ^{15}N labelled fertilizer requirements can be calculated as follows:

— the amount of fertilizer required per row (one lot) for a given treatment;
— the amount required per isotope plot (X lots, where X is the number of rows per plot) for a given treatment;
— the amount required per experimental treatment (Y lots, where Y is the number of rows per plot × replications);
— the sum of the amounts required per experiment is the total fertilizer requirement for all treatments.

With this information it is possible to further estimate the ^{15}N requirements and make a cost estimate based on recent bid quotations from commercial suppliers.

2.2.5.3.8. Application of the nitrogen-15 labelled materials

The application of ^{15}N labelled materials in the field has a profound influence on sampling procedures and experimental results. The procedures should be described in detail when publishing the data.

The required amount(s) of ^{15}N labelled fertilizer(s) for the experiment is calculated and necessary precautions are taken for the correct application of ^{15}N labelled source(s) in the field: source, time and method of application.

When utilizing the direct approach for FNUE studies, the ^{15}N should be applied in a form that reflects the standard practice to be tested (e.g. solid fertilizer). Therefore, in order to draw conclusions about fertilizerN uptake and recovery, the isotopically labelled fertilizer should be chemically (carrier) and physically (form) identical to the commercial fertilizer. Most fertilizers are applied as homogeneously as possible, on a per row or per plot basis, in solid, dry form, broadcast or banded, with or without incorporation. Other application practices are left up to the research team for the development of fertilizer management practices.

In the indirect approach, any ^{15}N labelled material (fertilizer, plant or soil) is applied as a tracer to 'label' the soil or, in other words, to establish an ^{15}N:^{14}N isotope ratio higher than natural abundance; therefore, uniform application is required [119, 123]. If small amounts of ^{15}N labelled fertilizers (often leftovers) from other experiments are available, spray application of labelled solution over the entire area may be preferred [75]. Another method that has been successfully employed in several tropical locations is to incorporate, in a confined plot area, ^{15}N labelled plant residues from previous isotopic experiments.

Many techniques, tools and equipment have been used to ensure uniform application of ^{15}N labelled source(s) [121, 124, 125]. When an ^{15}N labelled source is applied over standing vegetation or seedlings, immediate follow-up watering should take place to wash any residual ^{15}N from the vegetation.

2.2.5.3.9. Field observations

Field visits should be made regularly, to follow the development of the crop and any differences among treatments.

The experimental field book should be kept up to date, with detailed records of experimental designs and procedures, crop observations, planting, cultural practices and applications, weed and pest control, crop growth, changes in climatic conditions, etc. Details of harvesting and sampling procedures should also be recorded (see below).

2.2.5.3.10. Harvesting and sampling

This activity, though laborious and time consuming, is critical to the validity of the ^{15}N recovery data. It is necessary to plan in detail the sampling strategy, considering the objectives of the experiment and the parameters of evaluation [119].

Most FNUE studies include plant samplings for quantitative estimates of plant recovery of fertilizer N and to compare fertilizer management practices. Therefore, times should be set for sampling, to follow biomass produced and the total amount of nutrient taken up during the course of the experiment. Sometimes several sampling harvests are made within a single growth cycle of a crop or through several seasons in rotational/sequential experiments.

During a single-season experiment, the final harvest of the isotope plots should not be later than physiological maturity, to minimize leaf shedding, seed shattering and other physiological phenomena of advanced maturity, which greatly increase the experimental error. Remember that harvestable products (grain, root, tubers, etc.) should be collected from the yield plots at full maturity.

The harvested area normally comprises the inner part (two or three central rows for crops planted in rows) of the isotope plots (microplots), leaving the remaining plot area (extreme ends of the central rows and outer rows) as borders (Figs 14, 15).

The harvesting procedure consists of gathering all above ground plant material in the harvested area of the isotope plots and treating it as a sample. Avoid contamination of plant samples with labelled soil.

In studies of fertilizer N balance sheets, roots and soil must be sampled along with plants. The roots must be washed carefully. Details on soil sampling procedures can be found in the review by Saffigna [120].

Some practical considerations for plant harvesting and sampling techniques are:

— Plan detailed harvesting/sampling operations and allocate necessary resources. Whenever possible, visit the field in advance. Similarly, prepare in advance bags, labels and a field book to record results.
— Careful labelling and organization of samples per treatment and replication are essential.
— Before leaving the field, check that all samples have been collected.

2.2.5.3.11. Sample preparation

Sample preparation is an essential step in all isotope aided experiments, but often it is not given enough attention. The ultimate goal is to obtain a representative sample for chemical and isotopic analyses [126].

The two basic considerations for subsampling are:

— The size of the sample required for chemical and isotopic analyses is usually very small (10–1500 mg). However, the amount of harvested plant material is often bulky (several kilograms) and the entire sample is too large for processing.
— When approaching maturity, many annual crops show not only differences in physical consistency but also non-uniformity in ^{15}N content among plant organs, thus often requiring fractionation or separation into parts (reproductive and vegetative), e.g. shoots and spikes or panicles in cereals, shoots and pods in grain legumes, tops and roots including the crown in sugar beet, etc., to obtain a representative sample [126].

A diagrammatic representation of the steps that have to be followed in the sampling and sub-sampling procedures is shown in Fig. 16. Before harvesting an isotope aided experiment, the procedures to be followed must be established taking into account the type of information to be obtained in relation to the objectives of the experiment and the availability of resources (personnel, sample preparation equipment, transportation, funding, analytical facilities, etc.).

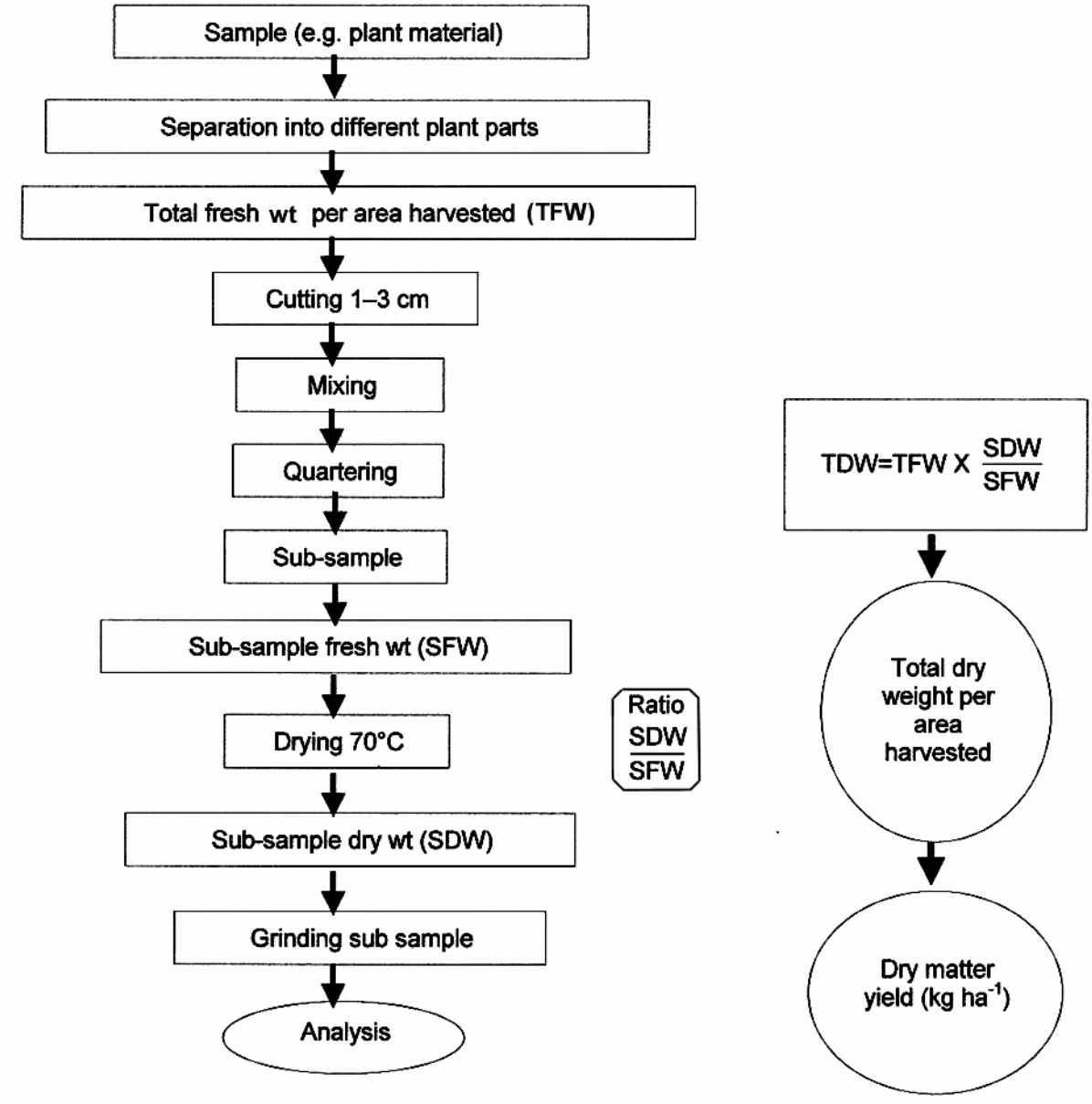

FIG. 16. Flow diagram showing sampling and subsampling.

In order to reduce the sample size, it is necessary to quarter the chopped sample by saving two opposite quarters, as shown in Fig. 17. Quartering is repeated until the sample size has decreased to 200–300 g fresh weight. The subsamples are weighed fresh and then placed in a draft circulation oven (for about 24 hours) at 70°C until constant dry weight is reached. The dry weight of the subsample is recorded and the final step is the grinding of the subsample to pass through a 1 mm sieve.

It is important that the weights of the total fresh sample (TFW) and its subsample (SFW) be taken within a short period, to avoid significant water loss between these two weighings; otherwise an inaccurate estimate of dry matter yield will be obtained. Particular care should be taken to obtain correct dry matter data when various plant parts (organs) are subsampled.

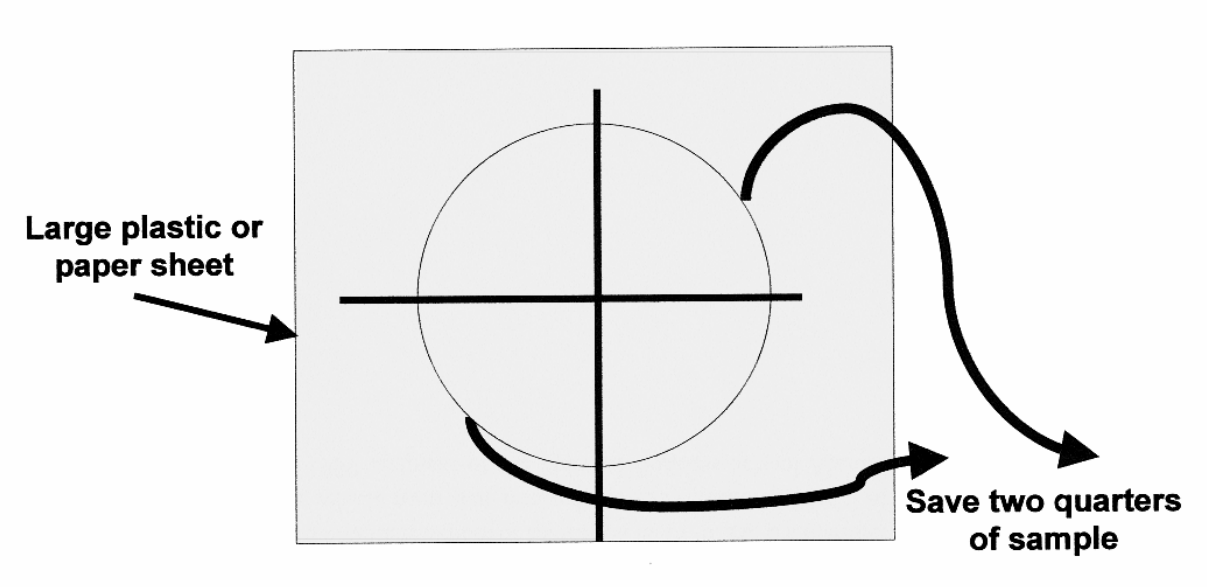

FIG. 17. Quartering procedure for subsampling.

Once the finely ground subsamples have been obtained, it is usually not necessary to analyse them (from plant parts) separately for FNUE studies. On the other hand, scientists must realize that, in the application of the isotopic method, the analytical process is laborious, time consuming and expensive. One should remember that the ultimate goal is to obtain a representative sample for chemical and isotopic analyses and fertilizer-N recovery data. Therefore, re-composing the plant samples of the experimental treatments (and replications) is recommended. Utilizing the ratios of the total dry matter weights of the samples, one can again obtain a composite sample (for each treatment and replication) by mixing the corresponding amounts of the dry subsamples (plant parts). It is important that these two subsamples be dried, finely ground and well mixed (homogenized). These composite samples, duly identified, will be sent to the laboratory for analysis. The remaining materials are retained as spares for additional analyses [126].

Exercise

Table XV shows sampling and subsampling data to be recorded in the field book. The procedure to obtain the composite sample is illustrated in the following example, utilizing the data from Table XV. The total dry weight (TDW) of the plant parts obtained through subsampling (SFW, SDW and SDW:SFW ratio) are: 528 g shoots and 119 g pods, thus the corresponding ratio of the plant parts in TDW is 4.44: 1. Therefore, we may take 8.88 g shoot

material and 2.0 g pod material and mix well to obtain a composite (representative) sample of treatment 2, replicate 1. Following this procedure, the total number of samples to be analysed will be halved, reducing costs and time without compromising the information obtained.

TABLE XV. SAMPLING AND SUBSAMPLING DATA

Sample treatment	Coding replication	Plant part	TFW[a] (g)	SFW[b] (g)	SDW[c] (g)	SDW:SFW ratio	TDW[d] (g)
2	1	Pod	850	270	37.8	0.140	119
2	1	Shoot	2,980	247	43.8	0.177	528

[a] Total fresh weight.

[b] Shoot fresh weight.

[c] Shoot dry weight.

[d] Total dry weight.

Sample preparation techniques are reported to be the main source of error in isotope aided experiments. The following precautions are necessary to obtain precise analytical data and facilitate the interpretation of results:

— Careful organization of samples during preparation procedures (chopping and grinding) is essential to avoid cross-contamination problems. This is done by starting routine sample processing with the samples of expected lowest ^{15}N enrichment followed by increasing ^{15}N enrichments.
— Proper identification of field samples (treatment, replication and plant part) coming from the harvested plots and of the obtained subsamples, which are sent to the laboratory for chemical and isotopic analyses. All of this information should be entered into the experimental field book.

When the subsamples are analysed elsewhere, the complete information should be provided in duplicate: one report included in the parcel and the other mailed separately to the laboratory rendering the analytical services, together with the plant subsamples and fertilizer standard(s) used in the experiment. Every sample should have proper identification (treatment, replication, plant part). It is the responsibility of the chief scientific investigator to prepare the reports, check the parcel contents for correctness and completeness, and to send them to the laboratory for analysis. Past experience with IAEA projects shows that many mistakes can arise if these precautions are neglected.

2.2.5.3.12. Total-N and nitrogen-15 analyses

In this laboratory phase, it is necessary to analyse the plant and soil samples for total N and for the ^{15}N:^{14}N ratio or ^{15}N abundance. All samples collected in the field must be properly codified by treatment and replication. Samples of ^{15}N fertilizer standards and labelled materials (solutions) used in the experiment must also be included [126].

The ^{15}N abundance (or the stable N isotope ratio, ^{15}N:^{14}N) is determined in N_2 gas generated from the samples, by either mass spectrometry or optical emission spectrometry. For procedures for the chemical and isotope measurement techniques, the reader is referred to reviews by Bremner [127, 128], Fiedler and Proksch [129], Hauck and Bremner [87], Buresh et al. [130] and Pruden et al. [131], to IAEA manuals [90, 91] and to chapters in the book by Knowles and Blackburn [76];

Major established laboratories have their own routine analytical and quality control procedures. It is advisable that all laboratory facilities performing these services participate in the annual intercomparison exercises organized by the IAEA Seibersdorf Laboratory to ensure compliance with quality standards and the production of good quality analytical data [90].

2.2.6. Calculations for experiments with nitrogen-15

2.2.6.1. Basic primary data

For all field and greenhouse experiments with ^{15}N labelled materials, the following basic primary data need to be recorded for each plot and treatment:

— Dry matter yield for the whole plant or subdivided into parts. This parameter is utilized to estimate the amounts of total N uptake, and is determined from the isotope plot. Agronomic yield data are obtained from the corresponding yield plots. Sometimes the dry matter yields are calculated separately by plant part (vegetative and reproductive, such as shoots and pods, straw and spikes, etc.) and then summed to obtain the total biomass or total dry matter produced by the crop at harvesting time.
— Total N concentration (% total N in dry matter) of the whole plant or plant parts, as in point one. This is done by chemical methods, e.g. Kjeldahl, or by dry combustion (Dumas).
— Plant % ^{15}N abundance, which is determined by emission or mass spectrometry.
— Fertilizer % ^{15}N abundance, which is determined by the same method and equipment as the plant samples.
— ^{15}N labelled fertilizer(s) used and N rate(s) of application.

2.2.6.2. Quantification of fertilizer nitrogen use efficiency

The first parameter to be determined when studying fertilizer N uptake by the istopic method is the N in the plant derived from the ^{15}N labelled fertilizer (Ndff). The information to calculate this parameter is obtained from the plant % ^{15}N abundance and fertilizer % ^{15}N abundance data (third and fourth points above). Nitrogen-15 abundance data must be converted into at.% ^{15}N excess by subtracting the natural abundance (0.3663 at.% ^{15}N) from the % ^{15}N abundance of the sample. Next, a series of calculations is made, as shown below.

The following calculations are needed to estimate FNUE in field experiments with ^{15}N labelled fertilizers:

— Nitrogen derived from the fertilizer (Ndff) and from the soil (Ndfs): isotopic parameters:

$$\%\text{Ndff} = \frac{\text{atom \% } ^{15}\text{N excess}_{\text{plant}}}{\text{atom \% } ^{15}\text{N excess}_{\text{fertilizer}}} \times 100$$

$$\%\text{Ndfs} = 100 - \%\text{Ndff}$$

— Biomass produced or dry matter yield per hectare (kg/ha):

$$\text{Dry matter yield} = \frac{\text{Fresh weight (kg)} \times 10{,}000\ (\text{m}^2/\text{ha})}{\text{harvested area (m}^2)} \times \frac{\text{SDW}}{\text{SFW}}$$

Where FW is the fresh weight of the harvested area and SFW and SDW are the subsample fresh and dry weights (in kg or g), respectively

— Total N uptake or N yield (kg/ha):

$$\text{N yield} = \frac{\text{Dry–matter yield (kg/ha)} \times \%\ \text{total N}}{100}$$

— Fertilizer N uptake or fertilizer N yield (kg/ha):

$$\text{Fertilizer N yield (FNU)} = \frac{\text{N yield (kg/ha)} \times \%\text{Ndff}}{100}$$

— Fertilizer N use efficiency (FNUE); fertilizer N recovery; real coefficient of utilization:

$$\%\text{FNUE} = \frac{\text{Fertilizer–N yield}}{\text{Applied–N rate}} \times 100$$

2.2.6.3. Calculation exercises for experiments with nitrogen-15

To illustrate the calculations to be made and the potential of using ^{15}N techniques, examples utilizing actual data from N fertilization experiments are presented below.

2.2.6.3.1. Example 1

Greenhouse experiment

In pots containing 2 kg soil, 100 mg N/kg as ammonium sulphate (1.39% ^{15}N abundance) was applied to flooded rice. At harvesting, the plant dry matter yield per pot was 14 g and the plant samples had 0.70% ^{15}N abundance and 2.2% total N.

Questions

(1) What fraction of N in the plant was derived from the fertilizer?
(2) What fraction of N in the plant was derived from the soil?
(3) What was the total N uptake or yield of the crop?
(4) What was the fertilizer N uptake?
(5) What was the FNUE or recovery by the crop?

Calculations and results (see fuller explanation in Example 2)

at.% ^{15}N excess plant = 0.70 – 0.37 = 0.33
at.% ^{15}N excess fertilizer = 1.39 – 0.37 = 1.02

(1) $\text{Ndff} = \frac{0.33}{1.02} = 0.324$ or %Ndff = 32.4

(2) Ndfs = 1 – 0.324 = 0.676 or %Ndfs = 67.6

(3) Total N uptake or yield of the crop = $\frac{14 \times 2.2}{100} = 0.308$ g

(4) Fertilizer N uptake by the crop = $\frac{0.308 \times 32.4}{100} = 0.1$ g or 100 mg

(5) FNUE or % recovery by the crop = $\frac{100}{200} \times 100 = 50$

2.2.6.3.2. Example 2

In a field experiment, 80 kg N/ha as labelled urea (1.37% ^{15}N abundance) was applied to a maize crop. Plants were harvested at tasseling. Dry matter yield was 4000 kg/ha, and the plant samples had 0.67% ^{15}N abundance and 3% total N.

Questions

(1) What fraction of N in the plant was derived from the fertilizer?
(2) What fraction of N in the plant was derived from the soil?
(3) What was the total N uptake or yield of the crop?
(4) What was the fertilizer N uptake?
(5) What was the FNUE or recovery by the crop?

Calculations and results

at.% ^{15}N excess plant = 0.67–0.37 = 0.30
at.% ^{15}N excess fertilizer = 1.37–0.37 = 1.00

(1) %N derived from the fertilizer: $\%Ndff = \frac{0.30}{1.00} \times 100 = 30$

(2) %N derived from the soil: Since the crop had only two sources of nutrients, the %N derived from the soil is obtained by difference as follows:
% Ndfs = 100 – %Ndff
100 – 30 = 70%

(3) N yield of the crop: The total amount of N contained in the crop during the experimental period is obtained by recording the dry matter yield and multiplying it by the % total N in the crop as follows:
$4000 \times \frac{3}{100} = 120$ kg N/ha

(4) Fertilizer N uptake by the crop: The amount of fertilizer N taken up by the crop is calculated by multiplying the total N yield by the fraction of Ndff:
$120 \times \frac{30}{100} = 36$ kg N/ha

(5) Fertilizer N use efficiency or recovery by the crop: The fraction of the fertilizer nutrient taken up by the plant in relation to the rate of fertilizer nutrient applied is commonly expressed as a percentage:
$FNUE = \frac{36}{80} \times 100 = 45\%$

2.2.6.3.3. Example 3

In a field experiment, 60 kg N/ha as ^{15}N labelled ammonium sulphate was applied to hybrid sorghum. The ^{15}N treated plots were harvested at the milk stage of grain development. The harvest consisted of gathering all above ground material in the harvesting area of the isotope plots and separating it into shoots and panicles. Fresh weights of both components were recorded. Adequate subsamples were taken, and chemical and isotopic analyses were performed on each subsample separately.

Question

What was the fertilizer N utilization of sorghum?

Calculations

As shown in Table XVI, the total N uptake and fertilizer N yield of each plant part have to be calculated separately. Thereafter, the data from the plant parts are summed to obtain the total N uptake or yield and total fertilizer N yield for the entire crop.

The next step is to estimate by back-calculation a weighted average %Ndff for the entire crop:

$$\%\text{Ndff} = \frac{25.5}{106} \times 100 = 24$$

TABLE XVI. CALCULATION SHEET FOR FERTILIZER NITROGEN USE EFFICIENCY

Plant part	Dry matter yield (t/ha)	Total N (%)	N yield or uptake (kg/ha)	Ndff (%)	FertilizerN yield (kg/ha)
Shoots	5.0	1.2	60	27	16.4
Panicles	2.2	2.1	46	20	9.10
Total	7.7		106		25.5

Finally, %FNUE is calculated using the total fertilizer N uptake or yield and the rate of fertilizer N application as $\frac{25.5}{60} \times 100 = 42.5\%$.

2.2.6.3.4. Example 4

The ^{15}N labelled single treatment fertility design is a variant of the direct approach to measure FNUE without plant–fertilizer interaction. In this example, the calculations to be made to assess the effect of N source (ammonium and nitrate in ammonium nitrate) and timing on fertilizer N recovery by winter wheat are shown.

Data from an experiment where $*NH_4NO_3$ and NH_4*NO_3 were applied to winter wheat in a two-split application, either in the autumn or spring, are given in Table XVII. The utilization of a balanced set of treatments where either $*NH_4$ or $*NO_3$ was labelled in the ammonium nitrate allowed calculation of the %Ndff, fertilizer N uptake and %FNUE as follows:

TABLE XVII. SINGLE TREATMENT FERTILITY EXPERIMENT

(data from the balanced set of treatments)

Table XVII. Single-treatment fertility experiment (data from the balanced set of treatments)

Source, N rate (kg/ha) and timing		Nitrogen yield			Ndff			Fertilizer N uptake		
Autumn	Spring	Grain	Straw	Total	Grain	Straw	Plant	Grain	Straw	Total
		(kg/ha)			(%)			(kg/ha)		
*NH_4NO_3										
30*	30	95	77	172	8.0	7.9	8.0	7.6	6.1	13.7
30	30*	94	78	172	10	9.0	9.8	9.8	7.0	16.8
30*+30*		94.5	77.5	172			18	17.4	13.1	30.5
NH_4*NO_3										
30*	30	94	78	172	10	11	10	9.6	8.3	17.9
30	30*	93	79	172	12	13	13	11.3	10.2	21.5
30*+30*		93.5	78.5	172			23	20.9	18.5	39.4

Calculations

The calculations of the parameters (total N yield, Ndff and fertilizer N yield) for each treatment are as shown above in the other examples. Results for each pair of treatments are given in Table XVII. The next step is to calculate the parameters for the combined treatment (30*+30*) by utilizing the data for the labelled treatments.

The total N yield is obtained by simple arithmetic averaging of the data for each pair of treatments. In this example for the *NH_4 NO_3 treatment, it was 94.5 and 77.5 kg/ha for grain and straw, respectively, whereas the average total N yield was 172 kg/ha. No major yield differences between each pair of treatments were expected because the applied fertilizer N rates were the same.

The total FNU data are obtained by addition of each pair of treatments. In this example for the *NH_4NO_3 treatment, it was 17.4 and 13.1 kg/ha for grain and straw, respectively, whereas the total FNU was 30.5 kg/ha.

A weighted average Ndff for the plant in the combined treatment is obtained by back-calculation from the total FNU data. In this example for the *NH_4NO_3 treatment, it is:

$\frac{30.5}{172} \times 100 = 17.7\%$.

Finally, the %FNUE can be estimated for each pair of treatments and for the combined set of treatments. In this example for the *NH_4NO_3 treatment, the %FNUE was obtained as follows:

$\frac{13.7}{30} \times 100 = 45.7\%$ and $\frac{16.8}{30} \times 100 = 56.0\%$ for the split-application treatments, and

$\frac{30.5}{60} \times 100 = 50.8\%$ for the combined set of treatments. The results for both sets of treatments are:

Timing and N rate		FNUE %		
Autumn	Spring	Grain	Straw	Total
*NH_4NO_3				
30*	30	25	20	46
30	30*	33	23	56
30*+30*				51
NH_4*NO_3				
30*	30	32	28	60
30	30*	38	34	72
30*+30*				66

These results show quantitatively that nitrate was a better ($\frac{66}{51}$ = 1.3 times) source of N for winter wheat than ammonium and that the spring application of either ammonium ($\frac{56}{46}$ = 1.2 times) or nitrate ($\frac{72}{60}$ = 1.2 times) resulted in greater FNUE than its respective autumn application. Additional conclusions can be drawn on grain and straw yield and N concentrations, depending on the objectives of the experiment.

2.2.6.3.5. Example 5

In field plots (4 × 4 m) with barley and maize, fertilizer N efficiency, as well as a balance of ^{15}N labelled fertilizer N recovery was investigated by Khanif et al. [122]. Barley received 50 kg N/ha as KNO_3, labelled with 5.99 at.% ^{15}N excess. Maize received 113 kg N/ha as KNO_3, labelled with 5.014 at.% ^{15}N excess. At harvest time, plant samples were taken from the central area of the fertilized plots: 3 × 3 m and 3.2 × 3.2 m of the barley and maize plot, respectively. Each barley plot was subdivided into nine subplots, while each maize plot was subdivided into six subplots. Plant samples, including the roots, and soil samples to 1 m depth (per 10 cm interval) were collected from each subplot. Roots from the top 25 cm were dug out and separated from the soil by wet sieving.

In order to find out whether the results obtained from the ^{15}N plot can be extrapolated to the whole field, an equal number of plant samples were taken at random from outside the ^{15}N plots. The means of N uptake of these samples were compared with the means of the ^{15}N samples using the t test, and their variances were compared using the two tailed F test.

Questions

(1) What fraction of N in the plant was derived from the fertilizer?
(2) What was the fertilizer N uptake by the crop?
(3) What fraction of fertilizer N was in the soil profile?
(4) What was the balance of applied fertilizer N?

Calculations and results

The dry matter yields and estimates of total N uptake by barley and maize are shown in Table XVIII. The at.% ^{15}N excess values in the plant parts (including roots) are given in Table XIX.

The %Ndff can be calculated from the at.% N excess in the plant and in the fertilizer (see above), leading to the data shown in Table XX. When these data are multiplied by the ratio of total N uptake to applied N rate (50 and 113 kg/ha for barley and maize, respectively), the % fertilizer N recovery (Table XX) can be calculated.

TABLE XVIII. DRY MATTER YIELD AND TOTAL NITROGEN UPTAKE BY PLANT PARTS OF BARLEY AND MAIZE

Component	Crop	Grain	Straw	Roots	Total
		(kg/ha)			
Dry-matter yield	Barley	2 540	2 680	620	5 840
	Maize	5 060	4 710	990	10 750
N uptake	Barley	65.5	35.1	7.2	108
	Maize	69.9	44.9	5.4	120

TABLE XIX. NITROGEN-15 ENRICHMENT IN PLANT PARTS OF BARLEY AND MAIZE

Crop	Grain	Straw	Roots
	(at.% ^{15}N excess)		
Barley	1.669	1.531	1.119
Maize	0.824	1.019	0.728

TABLE XX. NITROGEN DERIVED FROM FERTILIZER, AND FERTILIZER NITROGEN RECOVERY, IN PARTS OF BARLEY AND MAIZE

Plant part	Ndff (%)		Fertilizer N recovery (%)	
	Barley	Maize	Barley	Maize
Grain	28	16	37	10
Straw	26	20	18	8.1
Roots	19	15	2.7	0.70
Total			57	19

To make up a total balance of the applied labelled fertilizer N, the ^{15}N in the soil profile is calculated. From the at.% ^{15}N excess and the total N content in the different soil layers; the apparent or bulk soil density of each layer is needed to calculate the soil weight and the total N content of each layer. This amount is compared with the amount applied, to calculate the % recovery from the added ^{15}N labelled fertilizer (Table XXI). The labelled ^{15}N in the soil at sampling can be both mineral and organic N, or ^{15}N in non-recovered roots.

Finally, the percentages from the different soil layers are added to obtain the total fraction of labelled fertilizer N in the profile.

The balance of applied labelled fertilizer N (Table XXII) is calculated by adding up the % labelled fertilizer N found in the plants and in the soil; unaccounted-for N is the difference from 100%. Unaccounted-for N is that lost from the system by denitrification, nitrification, volatilization and leaching.

TABLE XXI. DISTRIBUTION OF FERTILIZER N IN THE SOIL AT HARVEST TIME

Soil depth (cm)	Barley (%)	Maize
0–10	18	3.5
10–20	6.7	2.1
20–30	3.8	2.2
30–40	0.97	5.3
40–50	3.2	20
50–60	0.00	23
60–70	0.00	10
70–80	0.00	2.2
80–90	0.00	0.43
90–100	0.00	0.00
Total	32	68

TABLE XXII. BALANCE OF APPLIED LABELLED FERTILIZER NITROGEN

Crop	Fertilizer N recovery (%)			Unaccounted-for N (%)
	Plant	Soil	Total	
Barley	57	33	90	10
Maize	19	68	87	13

In Table XXIII, data “A” on N uptake from the labelled plots, with nine and six repetitions for barley and maize, respectively, were compared with the same information (“B”) from the same number of subplots outside the labelled plot and scattered throughout the field. When the calculated t test and F test are smaller than the tabulated t test and F test for the same

number of repetitions, the obtained information on the labelled plots (limited size) can be extrapolated to the whole field.

TABLE XXIII. STATISTICAL ANALYSIS OF TOTAL NITROGEN UPTAKE IN THE NITROGEN-15 PLOTS (A) AND IN THE FIELD AS A WHOLE (B)

Crop	Parameter	Repetitions per plot	Mean (g N/rep.)	T	$t_{0.05}$	F	$F_{0.05}$
Barley	A	9	10.1	0.229	2.12	1.653	4.43
	B	9	9.91	–	–	–	–
Maize	A	6	17.2	2.012	2.23	1.107	7.18
	B	6	21.2	–	–	–	–

2.2.6.3.6. Example 6

Field experiments were carried out to assess the availability of *Azolla*-N and urea-N to rice, using both the direct and indirect approaches of the isotopic method [110].

Nitrogen-15 labelled urea was applied at 100 kg N/ha to all treatments, and unlabelled *Azolla* was applied at two rates (equivalent to 250 and 330 kg N/ha). The first treatment, without *Azolla,* was the standard or reference treatment. For more details on the experimental procedures consult Ref. [109]. This example, from Ref. [107], illustrates the use of the indirect approach, the so-called isotope dilution method (Table XXIV).

TABLE XXIV. RICE YIELD AND NITROGEN UPTAKE FROM UREA AND *AZOLLA* [109]

Urea (kgN/ha)	*Azolla* (kg N/ha)	DM yield (t/ha)	Total N yield (kg/ha)	Urea Ndff (%)	Urea FNUE (%)	*Azolla* NdfAz (%)	*Azolla* FNUE (%)
100	0	12.0a[a]	107a	25a	26a	–	–
100	250	21.5b	258b	14b	37b	44a	45a
100	330	21.9b	316c	11b	35b	56b	53a

[a] Means in the same column followed by the same letter are not statistically different at the 5% level.

The parameters %NdfAz, i.e. *Azolla*-N yield or uptake from the *Azolla*, and %FNUE-*Azolla*, were as follows:

$$\%\mathrm{NDFAz} = \left[1 - \frac{(\text{atom \% } {}^{15}\text{N excess of rice with } \textit{Azolla})}{(\text{atom \% excess of rice without } \textit{Azolla})}\right] \times 100 \quad (9)$$

or similarly,

$$\%\mathrm{NdfAz} = \left[1 - \frac{(\%\text{Ndff of rice with } \textit{Azolla})}{(\%\text{Ndff of rice without } \textit{Azolla})}\right] \times 100 \quad (10)$$

$$\textit{Azolla}\text{–N yield} = \frac{\%\text{NdfAz}}{100} \times \text{Total N yield} \quad (11)$$

$$\%\text{FNUE–}\textit{Azolla} = \frac{(\textit{Azolla}\text{–N yield})}{(\textit{Azolla}\text{–N applied})} \quad (12)$$

The application of these equations to the second treatment shown in Table XXIV is as follows:

$$\%\text{NdfAz} = \left(1 - \frac{14}{25}\right) \times 100 = 44$$

$$\textit{Azolla}\text{–N yield} = \frac{44}{100} \times 258 = 113.5 \text{ kg N/ha}$$

$$\%\text{FNUE–}\textit{Azolla} = \frac{113.5}{250} \times 100 = 45$$

Similar calculations were made for the third treatment shown in Table XXIV.

The recovery by rice of *Azolla*-N incorporated at the rate of 250 kg N/ha was found to be 45%, compared with 37% for the ^{15}N labelled urea applied at the rate of 100 kg N/ha in the same treatment. When 330 kg *Azolla*-N/ha were incorporated along with 100 kg urea-N/ha in the same treatment, the recovery of *Azolla*-N was much higher than that of ^{15}N-urea (53% vs. 35%). At both rates of *Azolla*-N incorporation, the recovery of urea-N was significantly increased in comparison with urea-N applied alone. Drymatter yield and N yield were significantly increased at both rates of *Azolla* incorporation as compared to the yields when urea alone was applied.

2.3 FACTORS AFFECTING EFFICIENCY AND LOSSES: ENVIRONMENTAL ISSUES RELATED TO NITROGEN FERTILIZER APPLICATION

2.3.1 Fertilizer efficiency

The efficiency of fertilizer sources is usually not very high. The use of ^{15}N labelled fertilizers allows direct and indirect quantification of use efficiency and of losses. A survey of some ^{15}N balance experiments in various parts of the world is shown in Table XXV.

From Table XXV it is clear that, although all levels of recovery and, consequently, of losses are found, plant uptake of fertilizer N is usually less than 50%. An important reason for the low efficiency is loss of the applied fertilizer from the plant–soil system. In order to minimize these losses, all effort should be made to increase the fertilizer N efficiency.

TABLE XXV. RECOVERY AND LOSSES (%) OF FERTILIZER N IN AGRICULTURAL SYSTEMS AS DETERMINED BY the ^{15}N BALANCE

(based on Ref. [132])

Plant	Country	Fertilizer[a]	Plant uptake (%)	Recovery plant+soil (%)	Loss (%)	Ref.
Barley	Canada	U	13–54	64–91	9–36	[133]
	Denmark	KN	53–62	86–90	10–14	[134]
Corn	Indonesia	U	32–36	42–50	50–58	[135]
	USA	AN	45–53	59–86	14–41	[136]
	USA	AS	24–60	49–100	0–51	[137]
Corn (no till)	USA	UAN	48	77	23	[138]
(alley crop)	Australia	AS	35–72	53–99	1–47	[139]
Cotton	Australia	U	29	57	43	[140]
Pasture	Australia	U	38–55	55–80	20–45	[141]
	Northern Ireland	AN/U	57–67	76–84	16–24	[142]
Potato	Belgium	AN	25–56	69–90	10–30	[143]
	UK	AN	49	61	39	[144]
Rice (flooded)	Australia	U	17	54	46	[145]
	China	ABC/U	21–27	28–37	63–72	[146]
	India	AS/USG	6–31	22–46	54–78	[147]
	Philippines	U	10–34	44–55	45–56	[148, 149]
	Thailand	U/USG	5–57	15–86	14–85	[150]
Rice (upland)	Indonesia	U	9–18	11–23	77–89	[135]
Rice (flooded)	Philippines	U	44	67	23	[151]
Sorghum	Australia	U	32–62	50–83	17–30	[152]
	India	U	30–55	72–94	6–28	[153]
Sugar cane	Australia	U	16–29	39–53	47–61	[154]
Sugarbeet	UK	AN	27	61	39	[144]
Wheat	Australia	AS/AN	38–45	60–91	9–40	[155]
	Belgium, France	KN	45	93	7	[156]
	USA	U/AN	34–49	70–72	28–30	[157, 158]
	UK	U	58	77	23	[159]
	Morocco	AN	32	52	48	[144]
Wheat–sorghum–fallow–wheat	USA	AS	31	93	7	[160]
		KN	63	89	11	[161]
Sunflower	Egypt	AS	22	6	72	[162]

[a] U = urea, KN = potassium nitrate, AN = ammonium nitrate, AS = ammonium sulphate, ABC = ammonium bicarbonate, USG = urea supergranules, UAN = urea-ammonium nitrate.

Only a limited number of studies have addressed the question of whether losses of N from synthetic fertilizers differ from those of organic origin such as manure [163]. The data in Table XXVI from ^{15}N experiments show that when inputs are properly managed, crops in rainfed systems usually recover more applied N from synthetic than from organic fertilizers; however, a higher proportion of applied N from organic sources generally remains in the soil at harvest. Therefore, the ranges of estimated losses are often rather similar. In lowland rice and irrigated systems, however, losses from fertilizer N can be substantially higher than those

from applied organic N. It should be mentioned that for the experiments with ^{15}N labelled legumes, only shoot material was taken into account.

TABLE XXVI. EXAMPLES OF THE FATE OF NITROGEN (ESTIMATED RANGE OF THE RECOVERY AND LOSSES OF APPLIED N) IN FIELD EXPERIMENTS INVOLVING THE APPLICATION OF ^{15}N ENRICHED FERTILIZERS OR LEGUME RESIDUES [163]

Source of N applied	Crop uptake	Recovery in soil	Total recovery (crop + soil)	Unrecovered (assumed loss)
	(% applied N)			
Rainfed cereal cropping[a]				
Fertilizer	16–51	19–38	54–84	16–46
Legume	9–19	58–83	64–85	15–36
Irrigated cotton[b]				
Fertilizer			4–17	83–96
Legume			62–82	18–38
Lowland rice[c]				
Fertilizer			61–65	35–39
Legume			87–93	7–13

[a] Wheat data from Canada [164] and Australia [165], maize and barley data from the USA [166], and maize data from Africa [167, 168].
[b] Data derived from Ref. [169].
[c] Data derived from Diekman et al. [170] and Becker et al. [171].

2.3.2. Factors affecting and measures to increase fertilizer nitrogen efficiency

Fertilizer N efficiency depends on type and amount of fertilizer, mode of application, and soil and crop characteristics as well as weather conditions.

In terms of management of N fertilizers, one commonly considers the spatial placement, and the mode and timing of the application. Foliar application is possible, but not discussed here as it is not commonly practiced. It is important to understand the efficiency parameters described in Section 2.4.1. For instance, AEN, the uptake efficiency (Section 2.2.1, Eq 5), and PEN, the transformation of N uptake into yield (Section 2.2.1, Eq 7), are both affected by environmental conditions and by crop (e.g. weeding, planting date, planting density) and water management practices (e.g. timing and method of irrigation, water harvesting method), as well as by fertilizer management practices. Consequently, to improve AEN by adapting N fertilizer management practices, it is essential to manage these other growth determining factors equally well. Secondly, as climate and other environmental factors (e.g. pest and disease pressures) change on a seasonal basis, interactions between those factors and fertilizer management practices are likely to occur frequently. This makes it relatively difficult to derive generally applicable rules for N fertilizer management. Alternatively, inclusion of agro-ecological and soil conditions in those fertilizer management rules could help in taking into account some of the above uncertainties.

The lowest fertilizer N recovery values have been found in Africa [29]. This may not be surprising, because of growth limiting factors such as lack of water, acid soils and/or

deficiencies of other nutrients such as P. It is generally accepted that fractional recovery decreases with increasing fertilizer N rate because of increased chances of N loss through run-off, erosion, leaching and gaseous emissions. These loss processes mainly depend on soil, climate and agricultural practices; a number of measures can be taken to minimize them and to increase N use efficiency:

— no excess inorganic or organic N fertilizer should be applied, and excess mineral N should be avoided during fallow periods;
— nitrogen fertilization should be synchronized with plant needs.

In practice, these conditions can be fulfilled through:

— application of fertilizer N at optimal rates, taking into consideration all N sources (applied as well as mineralized);
— when appropriate, fertilization should be split-applied, in order to be timed with the crop needs and development stage (multiple applications); when irrigation is used, there is opportunity to supply fertilizer N along with the irrigation water in accordance with crop requirements.
— avoiding fertilizer application outside the growing period and certainly before a fallow period;
— adjustment of the fertilization plan for conditions whereby unexpected losses might occur (e.g. excessive rainfall) or with deviations from the expected crop development;
— nitrogen uptake by the crop should be fostered by balanced fertilization with other nutrients; application techniques should be as professional as possible (e.g. precision farming, subsurface application, band or point application). For example, deep placement of urea or ammonium-containing fertilizers has long been known to substantially reduce N losses from paddies. Nitrogen loss is retarded both by placement in the reduced zone and by increasing the granule size, which gives a relatively smaller active surface area and a higher NH_4^+ concentration in the micro-site. Also, to avoid excessive ammonia losses and maximize N use efficiency, liquid manure (slurry) should be injected below the soil surface. The use of urease as well as nitrification inhibitors may retard the hydrolysis of urea and regulate nitrate accumulation. Under these conditions, fertilizer use efficiency can be increased and gaseous emissions decreased. Especially with regard to NH_3 volatilization, the following practical measures may help reduce losses [172]:
  - acidification;
  - irrigation at lower ambient temperature (night) and low wind speed, using short, narrow furrows to keep the exposed surface of flowing water to a minimum and to reduce turbulence;
  - use of fertilizer less susceptible to loss (e.g. loss rates are much lower when urea is applied in irrigation water in place of anhydrous NH_3);
  - application of N at later stages of crop growth, since foliar density influences wind speed close to the ground and shading reduces the temperature below the canopy.

2.3.3. Nitrogen losses and environmental consequences

If fertilizer N use efficiency is low, the non-efficient part is high and, consequently, so is the risk of loss from the plant–soil system. The most important pathways through which N is lost from terrestrial ecosystems are transformation dependent processes (nitrification, denitrification, nitrifier denitrification, volatilization), lack of synchronization and synlocation between the amount of fertilizer N and the demand for available N in the ecosystem (leaching, erosion), and the loss of dissolved organic N (DON).

Volatilization refers to the emission of ammonia (NH_3), while denitrification as well as nitrification are responsible for formation of nitric oxide (NO), nitrous oxide (N_2O) and molecular nitrogen (N_2). Denitrification by nitrifying organisms leads to synthesis of the same gaseous compounds. Chemodenitrification refers to the reduction of nitrate/nitrite with the same end products, but is not carried out by microorganisms. This non-biological process is important only in acid environments under specific conditions (presence of reduced elements, high levels of nitrite).

Mineralization immobilization turnover (MIT) as well as soil organic carbon (SOC) availability are major factors determining the effects of N fertilization on the environment. Temporary excess of supply over demand can occur on timescales from day to day, season to season, and for longer periods. Year to year variations in climate can drive temporary imbalances in N supply and demand, particularly in water limited systems.

Hedin et al. [173] suggested that losses of DON could represent an uncontrollable depletion of fixed N from natural/pristine ecosystems, one that could balance an eventually very low atmospheric N deposition. Losses of DON appear to be much less dependent on the N status of an ecosystem than is nitrate leaching.

In nitrifier denitrification, the microorganisms reduce nitrite via N_2O to N_2. Not much is known about the pathway. The differentiation between nitrification, nitrifier denitrification, denitrification and other sources of N_2O is usually based on response to various levels of acetylene (inhibiting nitrification and nitrifier denitrification) and oxygen (inhibiting nitrifier denitrification and denitrification) [168].

2.3.3.1. Ammonia volatilization

Global losses of N from the soil by NH_3 volatilization have been estimated at 54 Mt/year, of which 60– 75% is of anthropogenic origin [175, 176]. The background concentration in the atmosphere over land is about 2 μg NH_3/m^3. Ammonia is a metabolite that plants both emit to and absorb from the air. Net emissions of NH_3 from plants are of the order of 1– 5 kg N/ha [177]. The net NH_3 exchange within plants can influence fertilizer ^{15}N balance studies [178, 179]. Plants grown with ^{15}N enriched fertilizer tend to lose $^{15}NH_3$ and gain $^{14}NH_3$ even if the net flux is zero [180]. The actual impact of this exchange on N fertilizer studies is not always clear, although the NH_3 exchange would suggest that N fertilizer losses, estimated by ^{15}N balance, would be overestimated [178]. Overestimates of N loss by isotopic techniques can also be due to leaf drop before flowering, NH_3 emissions from decaying leaves or NH_3 exchange within the crop canopy. According to Mosier [177], the amount of NH_3 lost from crop vegetation ranges between 1 and 4% of fertilizer N applied and between 1 and 4% of the N present in the crop.

Emissions from plant residues during decomposition vary with N content and can be substantial from N-rich materials. No-till management can cause increased NH_3 volatilization [177]. An example of the impact of crop residue management on NH_3 loss is the practice of trash retention following green cane harvesting in sugar production [181].

On a global scale, livestock farming contributes about 70% of the total anthropogenic emission of NH_3. An excellent review of this subject was provided by Oenema et al. [182]. Studies with labelled manure are scarce (e.g., Refs [183–187]). Direct labelling of animal manure is possible by incorporating ^{15}N into the feed, but this is expensive, labour intensive and time consuming. Nevertheless it has been successfully done by Powell and Wu [188],

Powell et al. [189] and Munoz et al. [190, 191]. Another possibility is labelling the ammonium-N pool in slurry by adding a small amount of highly enriched NH_4^+. Moal et al. [178] demonstrated that the added N behaves as the slurry's endogenous NH_4^+. After application of ^{15}N labelled cow urine (1000 kg N/ha) to a pasture in a flooded irrigated-lysimeter study, Di et al. [187] found that 6.4–9.1% of the urine was lost by leaching, 29–39% was removed in the cut pasture, 46–48% remained in the soil and plant roots and less than 2% was lost by volatilization.

According to the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) [192], the dominant source is animal manure, and 30% of N in urine and dung can be lost as NH_3. The other major source is surface application of urea or ammonium bicarbonate, and to a lesser degree other ammonium-containing fertilizers. As urea is the most important N fertilizer globally, it may lead to important NH_3 losses (especially if surface applied) upon hydrolysis and subsequent pH rise in the vicinity of the urea prill. Ammonia losses depend on pH, soil moisture, soil temperature, soil composition, soil texture and structure, soil buffering capacity, weather conditions, etc. Global aspects and the influence of pH, $CaCO_3$ content, moisture content and temperature on NH_3 volatilization of some NH_4-containing chemical fertilizers are thoroughly discussed in, e.g., Refs [172, 193–195]. A summary of the influence of various parameters (pH, CaCO3, moisture content and temperature) on NH_3 volatilization from different types of N fertilizers is given in Table XXVII.

TABLE XXVII. INFLUENCE OF pH, $CaCO_3$ CONTENT, MOISTURE CONTENT AND TEMPERATURE ON NH_3 VOLATILIZATION OF VARIOUS NH_4-CONTAINING FERTILIZERS [193]

pH	$CaCO_3$ content	Moisture content	Temp.	Fertilizer			
				Ammonium sulphate	Ammonium nitrate	Urea	UAN solution[a]
L[b]	L	L	L	–[c]	–	+	+
L	L	L	H	–	–	++	++
L	L	H	L	–	–	±	±
L	L	H	H	–	–	+	+
H	L	L	L	+	±	+	±
H	L	L	H	++	+	++	+
H	L	H	L	±	±	±	±
H	L	H	H	+	±	+	±
H	H	L	L	++	+	++	+
H	H	L	H	++	+	++	+
H	H	H	L	+	±	+	±
H	H	H	H	++	±	++	+

[a] Urea ammonium nitrate solution: 50% urea + 50% NH_4NO_3.
[b] L: low; H: high. [c] Volatilization: – low; ± moderate; + high; ++ very high.

Urease inhibitors have been used to reduce ammonia volatilization. Rice et al. [196] reported 18–36% increases in irrigated and dryland corn yield, respectively, by applying n-butyl-thiophosphoric triamide (NBPT) with urea, compared to urea or ammonium nitrate alone.

Subsurface placement of manure and urea reduces NH_3 volatilization, but does not eliminate it. The most effective means of limiting volatilization is by injection (Table XXVIII). Slurry dilution also inhibits NH_3 losses drastically. The difference can be explained by the fact that the positive NH_4^+ ions of ammonium sulphate and ammonium nitrate are held by the highly negatively charged exchange complex. In the case of urea, the neutral molecule can evaporate from the soil surface. Ammonia volatilization is highest during the first two days after slurry application, after which it is rather limited. Table XXIX illustrates the influence of the application method on NH_3 volatilization [197].

In addition to economic consequences, NH_3 volatilization is indirectly responsible for acid precipitation. In the atmosphere, NH_3 reacts with sulphuric oxides, forming ammonium sulfate, which is deposited onto the land in precipitation. This ammonium is microbiologically transformed to nitrate, producing protons. As a result, the pH of the soil decreases.

2.3.3.2. Denitrification and nitrification

Denitrification, nitrification and nitrifier denitrification are responsible for the formation of N_2O, NO and N_2 [174, 198], of which N_2O has the strongest influence on the environment. It stimulates the greenhouse effect and affects the stratospheric ozone layer. Throughout the industrial era, the atmospheric concentration of N_2O has steadily increased. It is now 16% (46 ppb) higher than in 1750. In 1998, the concentration of N_2O amounted to 314 ppb. Between 1980 and 1998, it increased at a rate of 0.8 ppb/year, equal to about 0.25%/year, and is thought to be causing 5–6% of the enhanced greenhouse effect [199]. Nitric oxide (NO) quickly oxidizes to NO_2; these together are expressed as NO_X.

TABLE XXVIII. PER CENT LOSS OF FERTILIZER NITROGEN APPLIED AT 200 kg N/ha TO A CLAYEY SOIL AT THREE DEPTHS AT 16°C [193]

Fertilizer	Depth (cm)	N loss (%)
Ammonium sulphate	0	37
	2	3.8
	4	0.5
Ammonium nitrate	0	12
	2	1.3
	4	0.7
Urea	0	31
	2	6.1
	4	0.6
UAN solution[a]	0	20
	2	3.9
	4	0.5

[a] 50% urea + 50% NH_4NO_3.

TABLE XXIX. AMMONIA VOLATILIZATION FROM CATTLE OR PIG MANURE AS A FUNCTION OF APPLICATION METHOD ON GRASSLAND [197]

Method of application	Spring application		Summer application	
	CM[a]	PM[b]	CM	PM
	(% of applied NH_4^+-N)			
Surface application	30	27	100	69
Diluted (1:3)	8	15	33	34
Rained in (20 mm)	14	7	11	25
Injected	0.2	0	0.2	0.9
Acidified	0.5		12.5	

[a] Cattle manure.
b Pig manure.

Probably 0.5–0.8% of fertilizer N applied is emitted as NO [195, 200], and 0.8% as N_2O [195, 201, 202]. These values are significantly lower than with the application of manure. Intensification of arable agriculture and of animal husbandry has made more N available in the soil N cycle, increasing the potential for emission of nitrogen oxides. The relative percentages of NO and N_2O formation depend strongly on the moisture content of the soil. At water filled pore spaces (WFPS) below 50%, mainly NO is produced from nitrification. At between 50 and 80% WFPS, formation of N_2O from denitrification is important. From 75% up, the formation of N_2 by denitrification is dominant [203, 204]. Next to water content, the most important determining factors for N_2O formation are availability of N, temperature and decomposable organic matter [205].

In the presence of sunlight, NO_X reacts with volatile organic compounds from evaporated petrol and solvents, and from vegetation, to form tropospheric ozone, which is, even at low concentration, harmful to plants and human beings.

The major gaseous end product of denitrification is N_2, which is a loss to plant availability, but without negative environmental effects. The N_2O:N_2 ratio produced by denitrification depends on many environmental conditions. Generally, N_2 production becomes more important than N_2O production at increasing anaerobicity [206]. Denitrification loss of N is usually less than 15% of the fertilizer input and is more important on grassland and when manure is applied [207, 208]. Peoples et al. [172] reported losses of 1 kg N/ha·day under conditions of high soil NO_3^-, temperature and water content. A literature review by Meisinger and Randall [209] showed fertilizer N losses of 2–25% in well drained soils, compared to 6–55% in poorly drained soils. Global estimates of annual direct emissions of NO_X from N fertilizers or manure applied to grasslands and crops are given in Table XXX [163]. It should be noted that in addition to emissions as a direct result of applications, subsequent emissions can occur upon nitrate leaching and runoff, and ammonia volatilization.

A comprehensive review of techniques for N_2O emission determination has been published by the IAEA [210]. Identification of the N source (fertilizer N or soil N) responsible for gaseous N compounds is possible by applying ^{15}N-enriched fertilizers to soil and using a chamber cover to isolate the atmosphere above that soil for a designated time to determine the rate of change of ^{15}N atoms in the chamber atmosphere. For calculations, the reader should consult Mulvaney [211], Mulvaney and Boast [212] and the IAEA [210].

TABLE XXX. ESTIMATES OF GLOBAL EMISSIONS OF N_2O AND NO FROM NITROGEN FERTILIZERS OR MANURES APPLIED TO GRASSLANDS AND CROPS [163, 195]

Source/amount of N applied[a]	N_2O	NO
	(10^6 t N)	
Fertilizer/77.8	0.9 (1.2% of applied N)	0.6 (0.8% of applied N)
Manure/32.0	2.5 (7.8% of applied N)	1.4 (1.4% of applied N)

[a] Data collected for 1436 million ha of crops and 625 million ha of grassland receiving applications of either fertilizer N or manure in 1995. The estimated fluxes account only for the increased direct emissions due to addition of synthetic fertilizer or livestock manure.

When using ^{15}N techniques, it is prudent to conduct N balance measurements along with direct N gas flux measurements. The root zone of the plant should be enclosed within a cylinder so that plants outside the treated area cannot withdraw labelled N from it; the depth to which the cylinder is inserted is an important factor. If the whole root zone of the plant is not enclosed within the cylinder, the area around the treated site must also be sampled [213, 214].

In an experiment to quantify the relative importance of fertilizer N and soil N with respect to N_2O emissions, Linzmeier et al. [215] found that, of the total N_2O emission measured, 10–40% was attributable to fertilizer N and 60–90% originated from soil N. Through the use of labelled ammonium and nitrate, Laughlin and Stevens [216] found that, in grassland, soil fungi were more responsible for N_2O production than were bacteria.

Since the air spaces within rice plants are conduits for gaseous flow of N_2 and N_2O, plants must be included inside the measuring chamber [217]. For this type of experiment, micro-plots are established and N fertilizer containing 50–80 at.% ^{15}N is added to the soil. Addition can be effected by either injecting the appropriate amount of solution a few centimetres below the soil surface in a grid across the microplot [218], or by removing the top 10 cm of the microplot soil and mixing it with the ^{15}N fertilizer [214], or by banding the ^{15}N fertilizer a few centimetres to the side of the plant row at 10–15 cm below the soil surface within the microplot, or by applying ^{15}N directly into the floodwater for rice [219]. Details on gas collection and calculations can be found in Ref. [210].

2.3.3.3. Leaching

Both applied nitrate and nitrate formed via nitrification from manufactured NH_4^+ as well as from NH_4^+ from animal manure can leach from the rooting zone. This leached NO_3^- may be denitrified at other places and returned to the atmosphere. The amount and intensity of rainfall, quantity and frequency of irrigation, evaporation rate, temperature, soil texture and structure, type of land use, cropping and tillage practices, and the amount and form of fertilizer N all influence the amount of NO_3^- movement to groundwater and surface waters. Actually, nitrate in drinking water is of global environmental concern.

Even though some scientists doubt the adverse effects of dietary nitrate on human health [220, 221], there are other arguments for enforcing a reasonable limit for the nitrate level in ground and surface waters used as drinking supplies [222]. First, the nitrate limit in drinking water of 50 mg NO_3^-/L originates from very limited 1950s data, and links with methaemoglobin anaemia and cancer are either of minor importance or not scientifically proven at all [221]. Second, increasingly there are indications of beneficial effects of dietary nitrate [223]. A rise of the N content of ground and/or surface waters is a symptom of improper use of N fertilizers, inorganic as well as organic, and/or of poor agricultural management practices. In the European Union (EU), the Nitrate Directive (91/676/EEC) [224] and the Water Framework Directive [225] strive to attain reasonable ground and surface water quality in the near future in the EU. The main objective of the Nitrate Directive is "to reduce water pollution caused or induced by nitrates from agricultural sources and prevent further such pollution." The Water Framework Directive is much broader and has the objective of establishing a framework for the protection of inland surface waters, transitional waters, coastal waters and groundwater. It includes not only a reduction of pollution, but also the promotion of sustainable water use and mitigation of the effects of flooding and drought [226].

Crops with a restricted rooting depth and rooting distribution, or those harvested at high residual N, can cause important drainage losses during periods of excess rainfall. Examples are the possibly high amounts of N residual after potatoes and several vegetable crops. A number of N fertilizer efficiency and balance studies in the field as well as lysimeter studies using ^{15}N have quantified leaching of fertilizer N below the rooting zone [187]. Excess fertilization, excess rainfall or irrigation, unfavourable growing conditions, absence of crops, as well as soil texture and structure fostering water and nutrient movement can lead to high N loss, as found by experiments using labelled fertilizers [156, 227–230]. Therefore, to minimize leaching it is necessary to continuously improve scientifically based N fertilization recommendation schemes.

A number of leaching models are available and are discussed in Ref. [231]. Extensive literature concerning N management, leaching and groundwater quality exists, including that assembled by Follett et al. [232–234].

2.3.2.4. Nitrogen losses by run-off and erosion

In hilly areas, large amounts of N can be transported by surface run-off and erosion. Two important fractions can be distinguished: dissolved N and N adsorbed on sediment particles (particulate N).

In general, only small amounts of dissolved N are found in run-off water, as compared to other pathways of N loss. Indeed, because of its high solubility, the largest amounts of NO_3^--N are found in subsurface run-off and groundwater, while the upper layer of soil (0–5 cm) is depleted of soluble N. However, large amounts of particulate N can be transported by erosion of arable land. Because N, especially organic N and ammonium, is mainly adsorbed on clay-sized particles, the eroded sediment is often enriched in N, due to the selective erosion of finer particles at low erosion intensities. According to Sharpley [235], an enrichment ratio between the N content of the eroded sediment and the N content in situ, of between 1.5 and 3, is quite common. These losses are, together with leaching, responsible for eutrophication of surface waters. Increased input of plant nutrients results in excessive primary biomass production of algae and aquatic weeds. Nitrogen and P are responsible for algal growth, while the presence of silicon (Si) determines the composition of the algal community [194]. Depending on the

N/P/Si ratio, various species of microorganisms become important, some of them producing toxins. Run-off fertilizer N varies greatly with the application method and time of run-off events. These N losses can be reduced to a large extent by the use of grass filters [236]. In these riparian-zone buffer strips, dissolved N can be removed by denitrification, while particulate N is deposited.

2.4. WAYS OF IMPROVING THE EFFICIENCY OF FERTILIZER NITROGEN USE

2.4.1. Improving fertilizer efficiency

The present guidelines provide a historical perspective on the use of sources of N in agriculture and discuss challenges for sustainable intensification of agricultural production, likely to occur mainly in developing countries. In some developing countries, substantial increases in food production have been achieved with improved agricultural production systems, mainly through the development of high yielding varieties of cereal crops and increased use of agricultural inputs, in particular irrigation and fertilizers. However, these increases have often come at the expense of the natural resource base with adverse impacts on environmental quality, ecological sustainability of those agro-ecosystems, and human health. It is concluded that there is an urgent need for further improvement of FNUE.

Fertilizer N use efficiency, by definition, involves the amount of N taken up by a crop from that applied and is, therefore, dependent of the biomass (yield) produced. Also, for the farmer, the agronomic efficiency or the amount of harvestable product — cereal grain, potato tubers, tomato fruit, etc. — per kilogram of applied nutrient (N) is of paramount importance. Thus, all factors that affect biomass production (and economic yield) also influence FNUE. These factors are traditionally grouped into four main categories, namely:

- Soil factors: profile, effective depth, fertility status, organic carbon, total and mineralizable N, textural class, structure, pH, acidity, salinity/alkalinity, drainage, topography including position in the landscape, etc.
- Climate factors: rainfall records for a long period, annual total rainfall and distribution, dry spells, cold spells, frost occurrence and duration, average temperature monthly variations and distribution, relative humidity, sunny days and sunshine hours, etc.
- Crop factors: farming systems (including trees and perennial pastures), cropping systems (type and sequence of crops including green manures), crop varieties/cultivars (cycle, type, and yield potential), resistance to pests and diseases, genotype adaptation/tolerance to soil problems such as drought, acidity, salinity, etc.
- Farming/agronomic management practices that have been reported to affect FNUE:
  - time and type of tillage;
  - time and depth of planting (seed preparation);
  - appropriate crop rotation and crop variety;
  - use of soil amendments (liming in acid soils);
  - seed quality;
  - population density/plant spacing;
  - weed and pest control (weed infestation and insect attack);
  - balanced nutrient application (imbalanced fertilization);
  - combinations of fertilizers and nutrient sources;
  - rate, placement and timing of fertilizer N application;
  - water regime/irrigation methods/ irrigation management;
  - harvesting methods/efficiency.

Additionally, cultural and socioeconomic factors must also be considered for adoption and use of fertilizer practices [237].

Analysis and interpretation of the main factors affecting FNUE have been made by Moll et al. [238]. Most of the factors above are controllable by the farmer, to varying extents, during the cropping season. A number of controllable factors influencing fertilizer use efficiency are shown in Fig. 10. The main three controllable factors that reduce fertilizer use efficiency are unbalanced fertilization (20–50% reduction), inappropriate crop variety (20–40% reduction) and untimely sowing (20–40%). The latter is especially important in rainfed cropping systems [64]. However, some factors, particularly those related to climate, are beyond farmer control and can only be predicted. Inadequate climatic conditions are considered a serious constraint to fertilizer N recovery and sustainable crop production. Pilbeam [97] has made an analysis of the effects of climate on the recovery in crop and soil of ^{15}N labelled fertilizer applied to wheat under a range of environments worldwide. In wide tracts of arid and semi-arid regions of the world, dryland ecosystems are seriously affected by desertification and drought. In contrast, in tropical and subtropical areas, the soil–water regime can be very variable between and within seasons due to the occurrence of high rainfall and prolonged dry spells affecting soil and fertilizer N transformations, as well as crop growth and production. In humid temperate regions, a significant fraction of the residual fertilizer N can be lost during the autumn–winter period if the soil is left bare.

All these factors are site specific and very variable over time. Moreover, there are multiple and complex interactions among them for any specific situation, thus making appropriate fertilizer N recommendations difficult. Some interactions between these factors can produce strong synergistic effects on crop yields, but precautions must be taken to avoid mismanagement and negative effects on the quality of the products and on the environment, as well as economic losses.

Approaches and strategies to improve FNUE are examined below, with particular emphasis on the new integrated approach.

2.4.2. Classical approaches

The traditional approach involved determination of the relative influence of the factors mentioned above on FNUE by a given crop during a single growing season, with particular focus on agronomic efficiency and other economic indicators. In this context, soil testing/plant analysis and field evaluation fertilizer programmes (crop–response curves relating N rates and yields) were essential tools to provide advice/recommendations on fertilizer N application to farmers to achieve optimum yields. A wealth of information on this approach is available worldwide, but the usefulness of the data is limited because of site specificity. Nevertheless, selected data can be stored in databases for further analysis and provision of improved fertilizer recommendations. In recent years, such reports on “fertilizer use” have been prepared for some countries and are currently available on-line [15, 17].

Extensive networked research has been conducted using ^{15}N techniques to develop improved fertilizer management practices for major food grain crops (see Table II). The results of these projects are reported in a number of IAEA publications [69, 78–81]. The main results are summarized in an FAO Fertilizer Bulletin [3]. Factors influencing FNUE — fertilizer placement, timing, type of fertilizer, crop management practice (irrigation, planting density, cropping sequence, etc.), identification of N efficient genotypes, competition in mixed agricultural and natural ecosystems — have been studied [10, 63, 65, 68, 82–86].

In view of increased concern over ecological/environmental impacts of the application of fertilizers over the past two decades, the mechanisms/factors affecting losses of N from the soil–plant system have received attention to devise means of control/mitigation to protect the environment while increasing fertilizer N use efficiency. While some authors have directly measured losses of N from the soil–plant system, others have studied the fate of fertilizer N over several seasons or in cropping sequences to construct fertilizer N balances/budgets and to estimate the N losses in the unaccounted fraction [10, 85, 86].

In spite of all these efforts, FNUE values in agricultural systems at the global, regional and country levels are still low. Indeed, it is reported that FNUEs of cereals (main crop users) remain low in tropical and subtropical regions in spite of recent technological developments in agricultural production.

2.4.3. Integrated approach

In a first instance, enhancing sustainable intensification of agricultural production in a country/region requires proper use of the available natural resources in the main agro-ecological zones through land use planning at the catchment/watershed level to control soil and water quality [239, 240]. Member countries of the OECD such as those in the EU, the USA, Australia, New Zealand and Canada have adopted this approach, and others such as Brazil, India, South Africa and Chile are following it to assess environmental impacts of agriculture [241, 242]. From this assessment, the following strategies can be defined:

— agricultural intensification on the best arable land;
— adequate utilization of marginal lands;
— prevention of further soil degradation and restoration.

In preventing and reversing soil degradation, the main sustainability issues will be concerned with controlling erosion (by water and wind) and associated off-site impacts such as sedimentation and risks of eutrophication of surface water and contamination of groundwater [243, 244]. Significant amounts of nutrients, in particular N, can be removed from cropland due to water and wind erosion. Similarly, enhancing soil carbon sequestration (and soil N reserves) in cropland — to improve soil quality and productivity and mitigate the greenhouse effect — will also be a matter of concern [245].

To support future productivity gains on existing arable lands and income generation, novel soil and crop specific technologies should be developed, pilot tested and transferred in relatively short time frames. Issues such as improved crop rotations and cropping systems, soil and water conservation through crop residue management and conservation tillage, integrated nutrient management including improved fertilizer use efficiency, balanced nutrition, identification and development of crop germplasm with superior resource use efficiency and adaptation to harsh environments, and efficient water use practices need to be investigated. One of the main challenges is to identify appropriate integrated management practices best suited to particular agro-ecosystems, considering availability of inputs and socioeconomic conditions [33].

Thus, a more holistic and integrated approach to management of components of cropping systems in the main agro-ecological regions — ncluding socioeconomic factors — will be required to develop innovative technologies for sustainable crop production and to promote their adoption by farmers. This integrated approach will include soil, crop, nutrient and water management, since all these components are interactive and their combined effects are

potentially greater than their individual contributions. Due to the complex nature of the system, no simple solution should be expected [246]. Long term experiments will be required for the most thorough understanding of sustainability of the studied cropping/farming systems [99, 247].

Over the past decade, this integrated approach has been progressively adopted in the projects of the FAO/IAEA Programme, depending on locally available staffing skills and expertise, physical infrastructure and sustained financial resources. In addition, specialist research groups should commit to working together in close coordination at the national and regional levels. Substantial efforts are required in the creation (including training) and organization of individual groups. The coordination of participating groups (preparation of a work plan of the projec,; timely provision of equipment, supplies and services, search of financial resources, monitoring implementation and progress made, outreach activities, etc.) by the team leaders and the project coordinator is a demanding and continuous task [88]. The following section examines strategies and approaches for addressing issues related to N management in cropping systems.

2.4.3.1. Integrated plant nutrient management in cropping systems

The main aim of integrated plant nutrient management is to increase and sustain soil fertility in order to provide a sound basis for flexible food production systems that, within the constraints of soil and climate, can grow a wide range of crops to meet changing needs [31, 248]. It should be noted that balanced fertilization is required to avoid problems of depletion (mining) of any nutrients that are in short supply and imbalances and/or losses of nutrients that are available in excess. Improving nutrient use efficiencies for sustainable crop production in problem soils demands adoption of special and integrated management practices. Under Brazilian conditions, liming of tropical soils is the most common and effective practice for reducing acidity related problems, combined with the use of adapted crop genotypes [249]. In tropical and subtropical acid soils that are severely deficient in P, the use of phosphatic fertilizers, in particular reactive phosphate rocks, combined with P efficient genotypes, is a recommended sustainable practice [246]. In intensively cultivated areas there are reports of responses to other nutrients such as sulphur [250] and to micronutrients [251]. Thus, balanced fertilization (not only N) is required in intensively cultivated cropping systems.

The development and application of an integrated N management (INM) approach for agricultural production systems in developing countries implies the judicious use of all N sources available on a farm, or in a village or region. These include mineral N fertilizers and natural sources of nutrients, such as biological N_2 fixation (BNF), and animal and green manures, in combination with recycling of crop residues [30, 41, 252]. Rather than relying on mineral fertilizer N alone, it is important to include inputs of other N sources and strengthen N recycling processes within the farming/cropping systems [83, 253–256]. The proper combination of N sources will depend on the cropping system and site specific conditions. Isotopic tracers (^{15}N, ^{13}C, ^{32}P and ^{34}S) are used to understand nutrient process dynamics of the systems and to develop integrated nutrient management packages (manufactured mineral fertilizers and natural sources of nutrients such as BNF, phosphate rocks and organic manures) along with recycling of crop residues in predominant cropping systems [84, 88].

The efficient utilization of these technologies requires refined assessment of the N supply from the soil and of what materials are available locally as nutrient sources, their tailoring to specific cropping systems and the provision of guidelines for their application. This is

particularly the case for BNF, organic manures and crop residues utilized alone or in combination with mineral fertilizer N within a cropping system [33, 38, 112, 115, 257, 258].

2.4.3.2. Cropping systems and crop genotypes for efficient use of nitrogen

Type and sequence of crops influences the N dynamics of the system in terms of N input/output balance, recycling, residues, losses, etc., and the use efficiency of the applied N sources — in particular fertilizer N — due to the utilization of specific farm management practices [6].

The use of crop genotypes with superior nutrient use efficiency and tolerance of soil/climate stress conditions, along with optimizing the inputs of fertilizer N, BNF and efficient management of crop residues, are some of the approaches being used to develop sustainable and resilient cropping systems. The challenges are then:

— identification of best crop combinations suited to the agro-ecological zone and socio-economic conditions, and
— development and pilot-testing of technologies for efficient management of the available nutrient sources within each cropping system to address soil fertility constraints.

This integrated approach is being investigated using nuclear and related techniques in several projects of the Joint FAO/IAEA Division, such as in the tropical savannah acid soils of Africa and Latin America (maize and sorghum based cropping systems), the arid and semi-arid West African Sahel (millet based systems), tropical East Asia (rice based systems), subtropical East Asia (rice–wheat cropping systems) and degraded soils covering a wide range of agro-ecological regions (tree based cropping systems) [33]. International agricultural organizations (CGIAR centres and advanced research organizations) in cooperation with national institutes are implementing research projects utilizing this approach, targeting specific cropping systems.

In some agro-ecological zones, there is a need to consider inclusion of trees and perennial pastures/natural vegetation (and fallows) as components of sustainable annual cropping systems [255, 259].

As the agricultural frontier is likely to expand into marginal lands with harsh environments that contain fragile soils with lower productive capacity and higher risk of degradation, the use of plant genotypes with adequate yield potential, efficient in nutrient use and tolerant of soil and environmental stresses (drought, acidity, salinity, frost, etc.), will be of strategic importance [63, 260]. In this context, past research work has focused on identification of nutrient efficient species and/or cultivars [249, 261]. As increased N use efficiency is of particular importance to cereals, it has been studied mainly in rice [262, 263], maize [238, 264] and wheat [265]. Dilz [266] reviewed efficiency of uptake and utilization of fertilizer N by plants.

However, little or no information exists on the relative tolerance of selected crop genotypes in terms of superior N use efficiency under particular stress conditions (in harsh environments) such as aluminum toxicity in tropical acid soils (W. Horst and Ph. Monneveux, personal communications), high salt concentrations in salinity affected soils, drought occurrence in arid and semiarid areas, etc. This combined approach is becoming increasingly important in many international and national breeding programmes, i.e. making use of germ plasm diversity and enhanced breeding techniques [267–269]. The strategic integration of ^{15}N along with

molecular biology and classical breeding methods will foster rapid progress in the identification and development of superior genotypes [63, 88].

2.4.3.3. Improving fertilizer nitrogen use efficiency

It is important to note that, even in low productivity situations, the quantity of nutrients available for recycling via plant and animal residues is rarely sufficient to compensate for the amounts removed in agricultural products. Thus, mineral fertilizers have a key role in areas with low fertility soils where increased agricultural production is required. Mineral nutrients are the major contributor to ensuring sustainable crop production and maintaining soil productivity. Over the past 30 years, additional nutrients applied as fertilizers have been responsible for 55% of yield increases in developing countries [31]. Fertilizer use efficiency is an important factor that needs to be taken into consideration in agricultural production systems, as inefficient use of fertilizer inputs represents not only an environmental hazard but also a substantial economic loss. Generally, fertilizer use efficiency by crops is in the range of 50–70% for N, 10–25% for P and 50–60% for K [270, 271].

Management of fertilizer N is still a major problem in many cropping systems; when applied to soil, it undergoes various biochemical transformations resulting in substantial losses. This is especially true for wetland rice conditions, where fertilizer N recovery in most farmers' fields is only about 25–40%, as considerable N is lost to the atmosphere by ammonia volatilization and denitrification [272–275]. Extensive research has been conducted to evaluate the fate of applied fertilizer N under a range of agro-ecological conditions, and several strategies have been proposed to increase rice production through improved FNUE, such as incorporation of fertilizer before planting, deep placement of urea by machine or by hand, various split-application techniques, combined application of mineral fertilizers with organic manures, straw and green manures, application of urea fertilizers with urease and/or nitrification inhibitors and coated urea fertilizer [276, 277]. However, for various reasons, such as lack of machinery for transplanting and placement of fertilizers, inadequate water management practices for incorporation of fertilizers before transplanting, and inability to integrate proposed management strategies into local farming practices, farmer adoption of these technologies has not been as high as expected.

With regard to management practices, N fertilizer can be broadcast over the complete plot, banded along the planting line or spot applied near the planting hole. Spot application is usually done using a calibrated spoon, but urea supergranules are another option. For each of these practices, the fertilizer can be left on the surface or incorporated. Furthermore, due to its relatively high mobility, N fertilizer is commonly split over at least two applications, to better match the N uptake patterns of specific crops. For maize, for instance, two applications, 30–50% around planting time and 50–70% four to eight weeks later is a common practice.

Mughogho et al. [20] reported that, irrespective of the N source, broadcast applications outperformed band or point placement in the humid tropics, while point placement of urea was observed to be an inferior application method in the humid, subhumid and semi-arid zones. Concentrated placement of urea may have led to higher losses due to leaching (humid and subhumid zones) and/or ammonia volatilization (semi-arid zone). In all zones, a basal application of urea performed more poorly than split-applied urea, with an approximate relative yield reduction at the maximum yield of 9, 34 and 15% in the humid, subhumid and semi-arid zones, respectively. Urea needs to be incorporated, especially in the absence of

sufficient rain or irrigation, as under semi-arid conditions, since urea hydrolysis increases the pH of the surrounding soil (Fig. 18), with a high potential for ammonia volatilization.

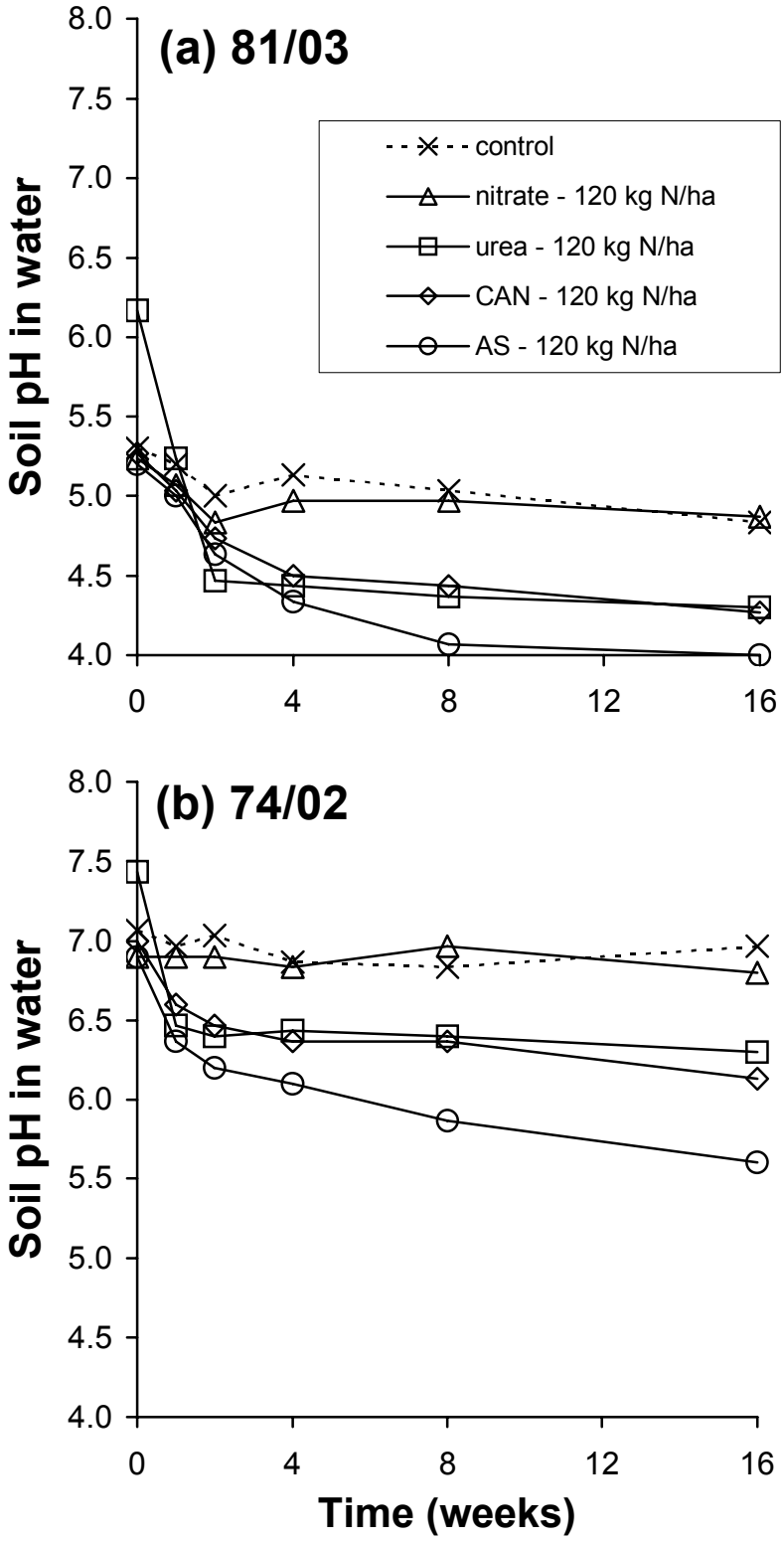

FIG. 18. Changes in soil pH after application of four sources of nitrogen fertilizer on tow soils from the West African moist savannah zone with varying initial soil pH. CAN: calcium ammonium nitrate; AS: ammonium sulphate. The nitrate fertilizer added was potassium nitrate [278].

To improve fertilizer N use efficiency, it is essential to identify promising fertilizer management practices that suit local soil and farming practices. For this purpose, it is necessary to obtain information on the issues mentioned above in the context of integrated management of the cropping system. The main strategy should be to accurately determine the N requirements and identify suitable management practices to enhance N use efficiency for the entire crop rotation, not for any single crop. Moreover, in the case of N, there is a need to gain refined information on dynamics/cycling processes and their implications for the agro-ecosystem. Also, it is necessary to assess the value of site specific crop/soil/fertilizer management practices designed to improve overall N use efficiency of the agro-ecosystem under study with the ultimate goal of enhancing sustainable intensification of agricultural production. The correct use of ^{15}N techniques has proven to be a valuable and cost effective tool for these purposes (see sections above).

Soil testing/plant analysis and field evaluation fertilizer programmes (crop–response curves relating N rates and yields) are essential tools to provide advice/recommendations on fertilizer N application to farmers to achieve optimum yields. This should also include a thorough knowledge and understanding of the relative influence of the various factors that affect FNUE in the study area. The development and implementation of an efficient and dynamic fertilizer N advice system requires trained staff, infrastructure and financial resources. Current developments focus on faster and more precise methods for recommending more exact doses of fertilizer N, and a better schedule of fertilizer N applications combined with more accurate methods of application including precision farming to synchronize plant demand and nutrient supply [279–281].

Another strategy utilized to increase FNUE while reducing environmental hazards is with controlled and slow release fertilizers [282–285]. In 1996/1997 the estimated world total consumption was rather small at about 560 000 tons. The largest share of these fertilizers is used for non-farm purposes (lawn care, golf courses, nurseries and landscaping), mainly in the USA, Canada and western Europe. Only about 10% is used for agriculture, mainly for cash crops such as vegetables and fruit production. Use of these formulations in agriculture has almost doubled over the past decade, and demand for them is increasing; however, their application to field crops is still limited due to high cost (three- to tenfold higher than a conventional commercial fertilizer), representing about 0.15% of the total worldwide fertilizer consumption in 1996/1997 [286]. Moreover, there is a lack of knowledge and awareness among farmers, agronomists and consumers in general of the potential benefits of their use to increase FNUE, sustain high yields and reduce adverse environmental impacts related to nutrient losses [282, 284, 287]. Another approach that has been utilized with variable success is the application of chemical products (inhibitors) that retard the soil biochemical reactions involving fertilizer N, such as urea hydrolysis and nitrification. However, it is difficult to predict the behaviour of these products during the cropping season and their effects on applied N [288, 289].

Future research should be focused on development of more site specific nutrient management practices for improving fertilizer N use efficiency, considering factors such as field variability of the indigenous N supply and season specific factors such as temporal variability in plant N status. Several simple, rapid monitoring techniques and tools, such as the use of leaf-colour charts [290], chlorophyll meters, portable electrodes and strips to determine nitrate N concentration in soil and plants can help monitor N levels during the growing season. In addition, there is potential to use advanced technologies such as precision farming and remote-sensing techniques, and modelling tools for N dynamics and impacts of water quality can contribute to achieving further increases in the efficiency of use of split applications of

fertilizer N in some areas of the world [281]. However, farmers in developing countries may be reluctant to conduct such field experimentation if not adequately supported by agricultural research and extension services.

In conclusion, there is a need to gain refined information on N cycling processes for proper definition of the specific constraints and the relative values of crop/soil/fertilizer management practices designed to improve overall N use efficiency of cropping systems, with the ultimate goal of enhancing sustainable intensification of agricultural production. A comprehensive approach to all of these strategies is being implemented by the SCOPE Nitrogen Fertilizer Rapid Assessment Project (NFRAP) with a regional focus, following a stepwise method towards the overall goal of the International Nitrogen Initiative, i.e. to develop sustainable N management for providing food and energy (food security) to the world, yet minimize release of N to environment (ecosystem security) [10, 28].

2.4.3.4. Carbon and nitrogen dynamics under conservation agriculture

One of the main objectives of conservation agriculture promoted by the FAO and other agricultural organizations is to provide adequate nutrients to crops through integrated management of available soil, water and biological resources combined with external inputs such as fertilizers. Currently, about 45 million ha are under conservation agriculture, mostly in North and South America [291]. Zero tillage and low tillage are gaining importance in many cropping systems and regions. For example, in rice–wheat cropping systems, locally manufactured seed drills are now being used to plant wheat immediately after harvesting rice, enabling wheat maturation three or four weeks earlier, before hot, dry weather sets in, thereby increasing crop productivity. The impacts of changes in land use, including conservation practices on soil properties, and in long term nutrient cycling and availability, in particular carbon sequestration, are the main tasks to be investigated in examining the sustainability of these systems [247, 291, 292]. Preliminary studies conducted in Chile showed that, over a period of three years, a positive N balance can be obtained by zero tillage practices with residue incorporation, compared with a negative N balance in conventional tillage with residue burning [293]. Similar studies comparing N dynamics under conventional and zero tillage in several cropping systems are ongoing in Argentina, Uruguay and savannah areas (Cerrado region) of Brazil [294]. More research is needed to understand changes in soil organic matter and their effects on C and N dynamics and availability, soil physical parameters and CO_2 emission, for identification and manipulation of conservation practices for efficient use of natural resources [294, 295].

2.4.3.5. Improved water management practices

Competition for scarce water resources is another major constraint to increasing agricultural production in developing countries. Chronic water shortages or droughts occur in more than 30 countries. Agriculture is by far the largest user of water, accounting for about 70% of withdrawals worldwide and 90% in low income developing countries [296]. Moreover, rapidly growing municipal and industrial water demands in developing countries will increase water scarcity for agriculture, which, with a continued slowdown in water investments, could be a serious threat to future growth in food production [297].

Water is the single most important factor limiting crop production in large areas of the world. If it is available in insufficient quantities, fertilizer and crop varieties with high yield potential are useless. Thus, in identifying promising cropping systems, including the use of fertilizers,

it is essential to focus on efficient water management practices to sustain crop production both in irrigated and rainfed conditions.

There is considerable synergy between irrigation water and fertilizer use; the yield increase resulting from the rational use of the two production factors together is greater than the yield increase from either alone. However, under irrigated agriculture there is a tendency to use excess water, resulting not only in waste of this costly resource but also in losses of N by leaching and even runoff, in particular under furrow irrigation. In intensively cultivated areas, irrigation water and fertilizer N mismanagement can lead to substantial N loadings affecting soil and water quality and ultimately to soil/water degradation due to waterlogging and salinization.

During the past decade, substantial progress in improving field-irrigation water use efficiency, while preventing and controlling environmental problems, has been made through the development and testing of several strategies, such as improving and refining irrigation scheduling at particular stages of crop development [298]; deficit-irrigation practices for both annual and perennial crops [299, 300]; and use of soil conditioners/ameliorants, such as gel-forming hydrophilic polymers, to increase the water holding capacity of the soil [301]. More recently, considerable developments in instrumentation have been achieved, using advanced electronics and software for better (fast, flexible, reliable and accurate) data acquisition, storage and processing. These developments have facilitated fertilizer/salinity and soil–water monitoring, establishment of refined water balances, and formulation of improved irrigation scheduling [300, 302–305]. The latest technologies allow near-continuous monitoring of in-field fertilizer/salinity and soil moisture status [306].

Through optimal scheduling of irrigation and fertilizer N application, substantial savings can be achieved in water use while improving fertilizer N use efficiency, thus increasing crop yields [299, 300]. For example, recent studies in Uzbekistan showed that by proper scheduling of irrigation, the water requirement of winter wheat per unit of crop yield was reduced by 25% and yields increased by 18–50% [307]. Moreover, the introduction of water saving techniques such as drip irrigation systems and fertigation (fertilizer applied in irrigation water) have opened up new possibilities for controlling supplies of water and fertilizer to crops by maintaining desired concentrations of nutrients in the root zone and better distribution of water in the soil [308], in particular in regions scarce in water, such as West Asia [304].

In rainfed farming systems, which occupy about 80% of arable land, water is the main factor limiting crop and livestock production [309–311]. Under dryland agriculture, FNUE depends on the success or failure of the crop. Due consideration should be given to improving water use efficiency through water harvesting and recycling [312] and strategic use of improved irrigation techniques such as fertigation [304, 308].

Selection of crops to optimize the use of residual moisture after harvesting the main crop, use of water harvesting techniques (local practices to increase the storage of rain water), inclusion of legumes or fallows, addition of manure and crop residues to improve soil structure and thereby increase infiltration of rainfall into the rooting zone and minimize evaporation losses, and improved placement and split application of fertilizer N, all have shown promising results in increasing crop productivity in semi-arid and arid regions [88, 153, 160, 311, 313–319].

Recent studies in Africa indicate that small scale irrigation has considerable scope for increasing and sustaining crop production. In many dryland areas, a single irrigation can

significantly influence FNUE and crop yields. A study in Kenya showed that simple and cost effective fertigation techniques could be developed for high-value crops (vegetables) [320]. Thus, simple irrigation schemes can help to improve food security by reducing the risk of crop failure by drought.

2.4.3.6. Socioeconomic factors and policy issues

In addition to technical considerations, a number of socioeconomic factors and policy issues need to be taken into account because they will ultimately determine the adoption and use of technologies by farmers.

Some of the most important factors that will determine whether N fertilizer use is attractive are within the economic domain, as fertilizer acquisition involves capital that needs to be recovered with a minimal amount of gain. The FAO Fertilizer Programme [89] used value:cost ratios (VCRs) to evaluate the economic benefits of fertilizer use, calculated as the value of the additional yield after fertilizer application divided by the cost of fertilizers to achieve this (Table XXXI). It is thereby assumed that technologies with VCRs below 2 are not attractive enough for farmers to evaluate and adopt.

In the example in Table XXXI, VCRs are higher than 2 at all application rates but decrease as application rates increase. It is assumed that 1 kg of maize can be sold at US$0.2/kg and that 1 kg of fertilizer costs US$0.625. Fertilizer above 40 kgN/ha is assumed to be split-applied with two applications, while the lowest rate is applied in single dose. Labour for one fertilizer application is estimated to cost US$5/ha.

TABLE XXXI. HYPOTHETICAL NITROGEN RESPONSE CURVE AND ECONOMIC CALCULATIONS (adapted from Ref. [321])

Component	N fertilizer application (kg N/ha)				
	0	40	80	120	160
Maize yield (kg/ha)	2000	2580	2930	3100	3190
Extra maize yield (kg/ha)	0	580	930	1100	1190
AEN[a] (kg/kg)	NA[b]	15	12	9	7
Value-to-cost ratio calculations:					
Value of additional yield (US$/ha))	0	116	186	220	238
Cost of fertilizer (US$/ha)	0	25	50	75	100
Value-to-cost ratio	NA[a]	4.6	3.7	2.9	2.4
Marginal analysis:					
Gross benefits (US$/ha)	400	516	586	620	638
Cost of fertilizer (US$/ha)	0	25	50	75	100
Cost of application (US$/ha)	0	5	10	10	10
Total variable costs (US$/ha)	0	30	60	85	110
Net benefit (US$/ha)	400	486	526	535	528
Marginal rate of return (%)	NA	287	133	36	<0

[a] Agronomic efficiency of N.
[b] Not applicable.

Marginal analysis calculates marginal rates of return between treatments, proceeding in steps from lower cost to next higher cost (Table XXXI), and compares those rates of return to the minimum rate of return acceptable to the farmer. It is assumed that farmers should be willing to adopt a treatment if the marginal rate of return of that treatment is higher than the minimum rate of return. The minimum rate of return, for the majority of situations, is between 50 and 100% and tends to be towards 100% for technologies that require new skills [321]. Rates of return above 100% are safe to recommend to farmers. In the example, application rates above 80 kg N/ha offer low or even negative rates of return, indicating that applying 80 kg N/ha would be the best recommendation. An important message resulting from economic analysis is that application rates that result in the highest yields (160 kg N/ha in Table XXXI) do not always make the best economic sense. More explanations on procedures to calculate economic benefits and their interpretation can be found in Ref. [321].

The above considerations are especially important in relation to farmers' resource endowments (e.g. access to cash for purchasing external inputs, access to labour). This has been shown to strongly affect the use of fertilizer [322], resulting in various fertilizer usages among households within a village. In Shinyalu, western Kenya, wealthier farmers apply 7–17 kg N/ha while the poorest farmers apply 0 –3 kg N/ha [323]. The method of N application also has consequences for labour requirements; incorporating fertilizer near the planting hole is more tedious and time consuming than broadcasting it on the soil surface.

The major constraints to adoption of technologies, such as lack of resources, farmers' perceptions and attitudes towards new technologies, local and macro-economic considerations and policy support for adoption of technologies, are some of the major factors that need to be examined.

Major factors have been found to determine adoption of technologies: ownership and property rights (or continuous possession) in order to ensure access to income flows; farm size; share of land under cropping and stocking rate (indicators of resource pressure); access to credit; off-farm income available for on-farm investment; the farming system; prices of and access to timely and adequate supplies of fertilizers and other variable inputs; and knowledge of and access to information on the use of fertilizers and agricultural production technology in general. These factors influence the time horizon (and implicitly the discount rate) for investment decisions and the degree of relative risk aversion of the farm households involved [237].

Despite considerable research and attention directed to the issues of technology adoption, a consensus has not yet developed regarding the social and economic conditions that lead farmers to take up new production practices. It is often unclear why some farmers adopt new technologies and others do not. Adoption of technological practices tends to be related to non-technological aspects. Therefore, agricultural programmes should emphasize the adoption of technological packages of practices rather than individual practices or a package containing all practices [324]. In the case of the integrated approach, this would include the best management practices for efficient use of N sources and other agricultural inputs available for the cropping system.

It is important to understand the attitudes of farmers towards adoption of new technologies. Enyong et al. [325] examined farmers' perceptions of the introduced soil fertility enhancing technologies in West Africa and concluded that farmers are knowledgeable of and practice technologies that encompass chemical fertilizer, crop rotation, phosphate rock application, and crop residue and farmyard manure addition to combat soil fertility decline. A number of

factors such as land use policies, labour resources, food security concerns, perceived profitability, contribution to sustainability and access to information influence their attitudes to, and rationales behind, adoption decisions. They also found that some of these factors are beyond farmers' control and concluded that a broad and integrated effort from research, extension and government, to promote the use of the soil fertility enhancing technologies in the region, is needed.

Although agriculture plays a vital role in the economy of developing countries, public sector expenditures on agricultural research are typically less than 0.5% of the value of agricultural production, compared with 2–5% in industrialized countries [326]. Thus, considering the present socioeconomic situation in many countries, policies supporting more resources for research and development of new and feasible local technologies should be provided, to achieve future sustainable agricultural production [327].

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CHAPTER 3

USE OF TRACER TECHNOLOGY IN BIOLOGICAL NITROGEN FIXATION RESEARCH

E.S. JENSEN,
Risø National Laboratory,
Roskilde,
Denmark

G. HARDARSON,
FAO/IAEA Agriculture and Biotechnology Laboratory,
Agency's Laboratories Seibersdorf,
International Atomic Energy Agency,
Vienna

M.B. PEOPLES
CSIRO Plant Industry,
Canberra,
Australia

This chapter discusses the use of tracer technology in the measurement and use of biological nitrogen fixation by leguminous crops. Whether grown as pulses for grain, as green manure, as pastures or as the tree components of agro-forestry systems, the value of leguminous crops lies in their ability to fix atmospheric N_2, thus reducing the use of expensive fertilizer-N and enhancing soil fertility. Nitrogen-fixing legumes provide the basis for developing sustainable farming systems that incorporate integrated nutrient management. Use of nitrogen-15 lends understanding of the dynamics and interactions between various pools in agricultural systems, including nitrogen fixation by legumes and utilization of soil and fertilizer nitrogen by crops in general, both in sole and mixed cropping systems (3.1). ^{15}N isotope dilution methodology has been found to be particularly useful to quantify and to enhance biological nitrogen fixation in leguminous crops (3.2). The final section of this chapter explores the data required to quantify a system's nitrogen balance, using crop legumes as an example, with particular emphasis on the methodologies that might be used to quantify the below ground contributions of nitrogen associated with roots and root nodules (3.3).

3.1. DYNAMICS OF LEGUME NITROGEN FIXATION IN INTERCROPS AND IN CROP ROTATIONS

E.S. JENSEN
Risø National Laboratory,
Roskilde,
Denmark

3.1.1. INTRODUCTION

Use of nitrogen-15 lends understanding of the dynamics and interactions between various pools in agricultural systems, including nitrogen fixation by legumes and utilization of soil and fertilizer nitrogen by crops in general. Biological nitrogen fixation in sole and intercropped legumes is discussed, as are intercrop design and methods of assessing possible yield benefits from intercropping. Advantages that accrue from inclusion of legumes, only some of which are linked with nitrogen tranfer, are discussed with particular reference to two case studies.

Legumes are integrated components of sustainable cropping systems. They contribute to diversity both in time and space via crop rotations and intercropping. The latter — simultaneous cultivation of more than one species in the same field [1] — often results in more efficient use of resources and more stable yields in problematic environments, and it often reduces problems with weeds [2], plant pathogens [3] and nitrogen (N) losses post grain legume harvest [4]. Legumes have three main functions in rotations as sole or intercrops:

— They supply protein and starch for animal feed and human nutrition to be used either on the farm or as a cash crop.
— They acquire N via symbiotic N_2 fixation and may contribute some of it to soil organic matter and to the N needs of succeeding and associated crops.
— They contribute non-N benefits in rotations, such as improved soil physical characteristics and mitigation of effects of pests, disease and weeds in succeeding crops.

The latter two functions are sometimes referred to as the rotational, the break-crop or the pre-crop effect [5]. The rotational effect is a complex interaction of factors related to nutrients, weeds, diseases, pest, soil structure, etc.

To improve N management and thereby reduce losses in rotations and intercrops, we must be able to determine N pools and processes. Some relevant research questions could be:

— How much N is fixed in sole (SC) compared to intercropped (IC) legumes?
— Is more N left in soil (spared) after a legume than after a cereal, and will this N be available for a succeeding crop?
— What is the pre-crop effect of a legume on the subsequent crop in a rotation, and how much of this effect is associated with N (N benefit) and with other effects (non-N benefit)?
— How much fertilizer N can be saved for the subsequent crop after legumes compared to other pre-crops?
— Is the capacity of the soil to supply N influenced by the pre-crop A_N-value?
— What is the effect of legume crop residues on the pre-crop effect?
— How much N does a legume contribute to an intercropped non-legume?
— What are the factors determining the sharing of soil and fertilizer N between a legume and a non-legume in an intercrop?
— How are the advantages of intercropping compared to sole cropping determined?
— What is the pre-crop effect of an intercrop compared to that of a sole crop?

To answer these questions and improve the efficiency of N use in cropping systems and reduce N losses from the agroecosystem, methods are required for determining the dynamics of N rotations and intercrops. In this context, the stable isotope ^{15}N is useful for giving more precise answers and for understanding the dynamics and interactions between various N pools in the nitrogen cycle. Some questions may be answered using total-N determinations and simple estimates.

3.1.2. BIOLOGICAL NITROGEN FIXATION IN SOLE AND INTERCROPPED LEGUMES

Intercropping legumes and non-legumes often results in a higher proportion of fixed N (%Ndfa) in the IC compared to the SC legume, because the non-legume is more competitive for soil N than the legume [6, 7]. However, the quantity of fixed N per unit area is typically less, due to other interactions such as competition for light, water, phosphorus (P), etc., but facilitative interactions, e.g. physical support of a vining legume, may enhance N_2 fixation.

The dynamics of symbiotic N_2 fixation in sole and intercropped legumes is determined by sequential sampling and application of ^{15}N methodologies [8]. The more frequent the sampling (e.g. ten times during the growth season), the better is the understanding of the dynamics as influenced by climate, soil and other plant growth factors, including competitive effects of intercropped species. Each sampling of a crop treatment should involve at least four replicate samples occupying more than $1m^2$.

Biological N_2 fixation (BNF) in intercrops is estimated using Eqs (5) or (10) in Ref. [8]. To determine N_2 fixation of legumes in intercrops using ^{15}N methodology, the reference crop should be the non-legume sole crop and not the non-legume(s) in the IC. This is because, in the IC, N from the legume may be deposited, mineralized and taken up in the associated non-legume, diluting the ^{15}N enrichment or natural abundance of the soil N. If the IC is used, BNF may be underestimated and the soil-N uptake overestimated (if the soil-N pool includes transferred N). Only if there is no sole non-legume crop established may the intercrop component be used.

Knowing the amount and proportion of N fixed in the legume, it is possible to determine the amount of N taken up from inorganic N sources (soil and fertilizer) by difference. This estimate may be of interest in relation to the determination of soil N use by the IC, which may differ from the soil N use by the SC.

Nitrogen fixation in IC legumes can also be determined by the difference method:

$$\text{BNF (kg N/ha)} = \text{total N legume}_{IC} + \text{total N non-legume}_{IC} - \text{total N non-legume}_{SC} \quad (1)$$

$$\%\text{Ndfa legume}_{IC} = \frac{\text{BNF}}{\text{Total N legume}_{IC}} \times 100 \quad (2)$$

It is assumed that the IC components take up the same amount of soil N as the non-legume sole crop. This may not be the case, because the competitive interaction between species may influence the growth rate and depth of the IC components [9]. Any N transfer from legume to non-legume is included in the BNF estimate.

3.1.3. INTERCROP DESIGN, YIELD ADVANTAGE, RESOURCE USE AND COMPETITIVE INTERACTIONS

Intercrops may be designed using a proportional replacement or an additive design [1], depending on the research objective. In the replacement design, crop density is maintained at a level resembling that of the sole crop. Example: mixed (IC components grown in the same row) 50%:50% bean and wheat intercrop; bean SC density = 40 plants/m^2; wheat SC density = 350 plants/m^2. Thus the IC replacement design means 20 bean plants + 175 wheat plants/m^2. The principle is to keep the number of plant units constant. One plant unit = 1 bean plant = 350/40 = 8.75 wheat plants. So one bean plant has to be 'replaced' with 8.75 wheat plants to maintain 'constant' density. It is also possible to design a 50%:50% row IC by establishing alternating rows (if the two species are grown at the same row distance) of the two species, or a 25%:75% bean-wheat intercrop establishing bean in every fourth row and maintaining the number of plants per metre of row as in the sole crop. If species are grown at different row distances in SC, it may be that one row of one species has to be replaced by two rows of the other species. In an added IC design, the overall plant density is not maintained. An example of an added design for a bean–wheat IC is 100% bean:25% wheat = 40 bean plants + 88 wheat plants/m^2.

The effects of intercropping compared to sole cropping can be evaluated by comparing total grain or biomass dry matter or total uptake of a given nutrient, compared to the sole crops. However, since dry matter production per unit nutrient uptake may differ for IC components, IC advantage is often determined in terms of the land equivalent ratio (LER) [1]. The LER is defined as the relative land area growing sole crops (using the same proportion of land for sole crops as the intercrop composition) which is required to produce the yields achieved when growing intercrops [1].

$$L_A = \frac{Y_{A,\,IC}}{Y_{A,\,SC}} \tag{3}$$

$$L_B = \frac{Y_{B,\,IC}}{Y_{B,\,SC}} \tag{4}$$

The LER for an intercrop of crop A and crop B is the sum of the partial LER values for crop A (L_A) and crop B (L_B) [1].

$$LER = L_A + L_B \tag{5}$$

A LER of >1 indicates an advantage from intercropping in terms of the use of environmental resources for plant growth compared to sole crops. When the LER is <1, resources are used more efficiently by sole crops than by intercrops. LER values can be determined for both replacement and added IC designs. They can also be calculated for nutrients; it may be of interest to compare the relative use of different nutrients by the IC component. Furthermore, the comparison of the partial LER values for different nutrients in an IC component may help to understand which nutrient is the most limiting. Typically, LER values for N in legume/non-legume ICs are higher than one, since N_2 fixation is a major contributor to the IC benefit.

In intercrop studies, it is also relevant to determine which species is the stronger competitor. The competitive strength of species A relative to species B is evaluated by calculating the competitive ratio CR_{AB} [10]. The ratio indicates the competitiveness of species A relative to species B:

$$CR_{AB} = \frac{L_A}{L_B} \times \frac{\%B \text{ in IC}}{\%A \text{ in IC}} \quad (6)$$

Values >1 indicate that species A is more competitive than B. Values <1 indicate that B is a stronger competitor than A.

3.1.4. TRANSFER OF NITROGEN FROM LEGUME TO NON-LEGUME IN INTERCROPS

Nitrogen is deposited from root exudates, dead roots and senesced nodules in the rhizosphere of legumes [11]. After mineralization of this N, it may be recycled to the legume or taken up by an intercrop component. Thus, in intercropping, the question of the ability of legumes to supply N to associated plants has triggered investigations back to the 1930s, many involving the role of mycorrhiza in the transfer [12, 13]. There is evidence that N can be transferred in annual crops, but usually the amount is small [14]. In perennial forage crops such as grass/clover mixtures, the transfer from clover may be significant after the first production year [15]. Transfer is usually small in annual crops because rhizodeposition may occur too late to be of significance.

The transfer of N from legume to non-legume may be determined directly or indirectly using ^{15}N methodology. In direct ^{15}N methods, the legume is labelled with ^{15}N without contaminating the soil during the labelling period. Tracer detected in the associated plant is evidence of N transfer. A number of ^{15}N methods have been developed, including N_2 labelling (e.g., Ref. [16]), foliar labelling (e.g., Ref. [17]), stem labelling by a cotton-wick method [18] and split-root labelling [14, 19]; see also Ref. [11]. Using these methods it is assumed that the ^{15}N tracer is distributed homogeneously within the legume. Oghoghorie and Pate [20] showed that some of the nitrate and symbiotically fixed N that is transported to the shoot is translocated back to the root and nodules. The longer the period of the growth cycle during which the legume is labelled, the better is the quantitative estimate. Thus, continuous methods such as $^{15}N_2$, cotton-wick and split-root labelling are preferable to pulse labelling methods, which are useful for detecting transfer.

When harvesting plants, it is essential to avoid contamination. Thus it is hardly possible to use the roots of ICs if a mesh has not separated them [14]. Mesh may also be used for compartmentalization to study effects of mycorrhiza [12, 13]. If roots intermingle, only the shoot materials should be used for isotopic analysis and estimations of transfer. If legume root material can be isolated (e.g. due to presence of root nodules), it is useful to obtain their isotope enrichment for the calculations. Nitrogen transfer from non-legume to legume has been reported [13].

Two direct approaches are available for calculating transfer: the N distribution [21] and donor root enrichment methods [17]. An indirect ^{15}N method is also available for determining apparent transfer of N in ICs.

3.1.4.1. Nitrogen distribution method

In this method, labelled N contents (g/pot or kg/ha) of the legume and receiver plants are calculated using the following equation:

$$^{15}\text{N-labelled N content}_{\text{plant}} = \frac{\text{atom\%}^{15}\text{N excess}_{\text{plant}}}{\text{atom\%}^{15}\text{N excess}_{\text{labelled N}}} \times \text{total N}_{\text{plant}} \quad (7)$$

Then the %N transfer can be calculated as follows:

$$\%N_{transfer} = \frac{^{15}N\text{–labelled N content}_{receiver}}{^{15}N\text{–labelled N content}_{receiver} + ^{15}N\text{–labelled N content}_{legume}} \times 100 \quad (8)$$

The amount (g/pot or kg N/ha) of N transferred from the legume is determined from the following equation:

$$N_{transfer} = \frac{\%N_{transfer} \times \text{total N}}{100 - \%N_{transfer}} \quad (9)$$

The proportion of N in the receiver plant derived from transfer (%Ndft) can be calculated as follows:

$$\%Ndft = \frac{N_{transfer}}{N_{receiver}} \times 100 \quad (10)$$

3.1.4.2. Legume root enrichment method

If it is possible to separate and analyse a representative root sample of the labelled legume, it is possible also to use the legume root enrichment method. Assuming that the N deposited in the rhizosphere and subsequently transferred has the same enrichment as the legume root when the plant is harvested, it is possible to determine the %N that the receiver plant derived from transfer ($\%Ndft_r$):

$$\%Ndft_r = \frac{atom\%^{15}N\text{ excess}_{receiver}}{atom\%^{15}N\text{ excess}_{legume\,root}} \times 100 \quad (11)$$

The amount of N transferred ($N_{transfer}$) and the proportion of legume N transferred ($\%N_{transfer}$) can then be calculated by rearranging Eqs (8) and (9).

Jensen [14] compared the two calculation methods in a series of experiments where legume roots could be isolated. In three short term studies with continuous labelling (up to 45 days), legume root enrichment estimates were twice as high as the N distribution estimates. In a 70 day labelling experiment, there was no significant difference between the two methods.

3.1.4.3. Indirect nitrogen-15 method

This method for determining apparent N transfer in intercrops is based on labelling of the soil in the IC and the sole crop of the receiver plant. The assumption is: if N is deposited by the legume and taken up in the associated receiver plant, the ^{15}N abundance of the latter will be lower than that in the sole crop of the receiver plant. The soil may be labelled using organic or inorganic ^{15}N materials, and even soil at relatively high natural abundance (δ >10) may be used. When harvesting, it is essential to keep the IC components separate, which is why roots cannot be included in estimates.

When the isotopic data are obtained, the first analytic step is to carry out an analysis of variance on the at.% ^{15}N excess of the receiver in IC and as SC. If the analysis of variance shows significance between the two cropping situations, the next step is to calculate the apparent transfer. The ANOVA is necessary since, especially in the field, plant enrichment may be quite variable [6]. The following equation is used to calculate the %Ndft in the receiver plant:

$$\%\text{Ndft} = \left[1 - \frac{\text{atom}\%^{15}\text{N excess}_{\text{IC}}}{\text{atom}\%^{15}\text{N excess}_{\text{SC}}}\right] \times 100 \quad (12)$$

The amount (g/pot or kg N/ha) and proportion of legume N that are transferred are then calculated by rearranging Eqs () and (9).

The resolution of the indirect methodology is much less than that of the direct methods, because in the former the pool of non-labelled N transferred is quite small compared to the large pool of labelled soil N. Secondly, as mentioned above, especially in the field, the ^{15}N enrichment of the soil may be highly variable. Thirdly, the rooting pattern and depth may change for the receiver plant when it is in competition with the legume [9]; for example, higher uptake of non-labelled soil N from a deeper layer may be misinterpreted as N transfer. Fourthly, different soil-N uptake patterns of the non-legume in IC and SC as a result of competitive interactions in IC in connection with mineralization–immobilization turnover of labelled N in the soil may also result in incorrect interpretations (see Section 8.1). In several indirect studies it has been shown that the amount of N transfer fluctuated during the growing season [6, 22] and did not increase steadily as would be expected.

3.1.5. RESIDUAL NITROGEN FOLLOWING LEGUMES IN ROTATION

It is often found that more soil inorganic N is available at the time of harvesting a grain legume than with a cereal [23, 24]. With forage legumes, the soil inorganic N concentration is usually low during growth, whereas, after ploughing down, a flush of net N mineralization may occur. The higher levels of soil inorganic N after grain legumes compared to cereals have been interpreted as the legume being unable to recover available soil N, either due to shallow root systems or symbiotic N_2 fixation resulting in lower requirement for mineral N. Thus, this has been termed the "N sparing or N conserving" effect (e.g., Ref. [24]). Indeed, this is most likely to be the case in deep soil layers, but an "N sparing" effect observed in the plough layer may be associated with differences in mineralization–immobilization turnover of N. Since root residues of legumes usually have a narrower C:N ratio than, e.g., cereals, it is likely that net mineralization may start earlier under the former. This effect can hardly be called sparing. Rhizodeposition of materials with a low C:N during the maturation phases of the legume is likely to enhance these effects (see Ref. [11]).

The soil inorganic N following a legume is determined by sampling using an auger, e.g. of 2.5 cm diameter, and many (more than ten per m^2) replicate cores per plot, extraction, and determination of nitrate and ammonium N using conventional methods [25].

3.1.6. LEGUME NITROGEN CONTRIBUTIONS TO SUCCEEDING CROPS

In crop rotation, legumes contribute benefits, only some of which are N associated. To understand how legumes influence N availability for the subsequent crops, we must separate N and non-N benefits. In some studies the preceding-legume effect was determined by comparison with a reference crop, which is also used for measuring the effect, e.g. comparing pea and wheat and measuring the effect of each in a following wheat. In such a case, the N benefit and non-N benefit are merged. By choosing another 'break' crop in order to minimize diseases of wheat, e.g. oilseed rape, any preceding-crop effect results mainly from N. The N benefit can be further separated into effects due to N 'sparing' and those due to N fixed in the legume [24]. Senaratne and Hardarson [26] developed a method to distinguish the effect of N from N mineralized from crop residues. Chalk et al. [24] first determined N uptake from soil,

fertilizer and N_2 fixation using ^{15}N dilution techniques in lupin, oilseed rape, ryegrass and wheat. The amount of soil N not used by the lupin was found to vary between 11.5 and 18.5 g/m^2, by differences between the uptake in the lupin and in the non-legume species. Plots grown to the same species, but without label, were subsequently labelled with inorganic ^{15}N, and barley crops were established after each of the four species. The actual N benefit from lupin to barley as compared to barley succeeding wheat was only 3.4 g N/m^2. The amount of N in barley derived from fixation was then determined in barley after lupin, with barley after wheat as the reference, assuming that the fixed N contributed from lupin would dilute the soil N pool for the succeeding barley. It was found that 1.6 g N/m^2 were derived from fixed N, and since the total N benefit was 3.4, the 'spared' N was estimated to be 1.8 g N/m^2 [24]. The %N derived from fixation in the succeeding (%Ndfa$_{suc}$) crop was determined using the following equation:

$$\%\text{Ndfa}_{suc} = \left[1 - \frac{\text{atom\%}^{15}\text{N excess barley}_{lupin}}{\text{atom\%}^{15}\text{N excess barley}_{wheat}}\right] \times 100 \quad (13)$$

where

at.%^{15}N excess barley$_{lupin}$ and at.%^{15}N excess barley$_{wheat}$ are the ^{15}N enrichments in barley crops following lupin and wheat, respectively.

Senaratne and Hardarson [26] compared pea and faba bean with barley, following them with sorghum. They found that the legumes accumulated less soil N than did barley. Then they added ^{15}N to plots with and without addition of above ground residues. Thus they were able to calculate the A_N value in the following sorghum. The A_N value indicates the N supplying power of the soil in relation to the availability of fertilizer N. The reason for calculating the A_N value was to obtain a measure of available soil N at a reasonable level of fertility; otherwise the total N accumulation of the succeeding crop can be used [26].

The A_N value can be used to determine the capacity of the soil to supply N at a realistic level of N supply and in fertilizer equivalents. It can be determined in plots with and without preceding legume cultivation. Nitrogen-15 labelled fertilizer is applied, and from the isotopic composition of the harvested crop, the A_N value of the soil is determined. Senaratne and Hardarson [26] found significantly higher A_N values after pea and faba bean than after barley. The estimated contribution from the legume residues (%Nfr), using sorghum after barley as the reference, was calculated according to Eq. (14):

$$\%\text{Ndfr}_{suc} = \left[1 - \frac{\text{atom\%}^{15}\text{N excess barley}_{legume}}{\text{atom\%}^{15}\text{N excess barley}_{barley}}\right] \times 100 \quad (14)$$

The assumption is that the residues (root and straw) will dilute the ^{15}N enrichment in proportion to the amount of residue N mineralized. Since the soil contained different amounts of unlabelled soil N at the establishment as a consequence of the previous crop, it would not be possible to determine the relative contributions of residue and 'spared' N. Senaratne and Hardarson [26] also observed that higher N uptake after the legumes compared to the cereal was not only a question of carry-over of 'spared' and residue N, but also of the succeeding crops being able to take up more soil N, perhaps because they were more healthy, indicating an interaction between N and non-N benefits.

The A_N value is determined from the %N derived from the fertilizer N (%Ndff) in the crop and the amount of fertilizer N added [27]:

$$\%\text{Ndff} = \frac{\text{atom}\%^{15}\text{N excess}_{\text{crop}}}{\text{atom}\%^{15}\text{N excess}_{\text{fertilizer}}} \times 100 \quad (15)$$

$$A_N = \frac{(100 - \%\text{Ndff}}{\%\text{Ndff}} \times \text{Amount of fertilizer N (kg N/ha)} \quad (16)$$

It is also possible to separate the residue-N benefit by using ^{15}N labelled crop residues and, in this way, determine their contribution, or by simply removing the above ground residues. In many studies on the effects of legumes on succeeding crops, scientist have expressed effects on the succeeding crop in fertilizer equivalents. This is done by growing the crop succeeding the legume and the reference crop at a series of fertilizer N levels (e.g. 0, 50, 100, 150 kg N/ha) (e.g., Refs [23, 28, 29]). From the two curves it is possible to determine at a given yield level how much extra N-fertilizer equivalents would be required after the reference to obtain the same yield as after the legume. Typical recovery of 1 kg fertilizer N on the soil used in the experiments by Jensen and Haahr [23] was 0.5–0.8 kg N/ha, determined by ^{15}N methodology.

As an example, we can take data from Jensen and Haahr [23], who used a fertilizer response design and oats as a reference crop to pea, and then combine the data with those of Jensen [30] using ^{15}N labelled crop residues. The typical preceding crop effect of pea was 30 kg N/ha in fertilizer equivalents for winter wheat and spring sown crops after pea. The actual uptake of N from 30 kg N/ha fertilizer — assuming a fertilizer recovery of 50% — would be 15 kg N/ha. This is consistent with reported recoveries of N from crop residues in a succeeding winter cereal of approximately 10–12 kg N/ha [30]. Thus, it indicates that in winter cereals, which are weak in the ability to take up N in the autumn, the N benefit is explained in terms of N released from residues. The N requirement of winter oilseed rape in the autumn is much higher than that of winter wheat. This was confirmed by Jensen and Haahr [23], who showed that the value of pea compared to oats, measured in winter oilseed rape, was 30–60 kg N/ha in N fertilizer equivalent to oats and that the residue N uptake from pea, in winter oilseed rape, was about 15–20 kg N/ha. Thus a major part of the N benefit was due to the residues, but slightly more was derived from deeper soil N after oats and pea [23].

Legume crop residues benefit soil fertility in the longer term. Studies on the turnover of ^{15}N labelled pea residues (see Ref. [31]) showed that after 16 years of decomposition, about 20% of the residue N was still present in the more recalcitrant fractions of the soil organic matter and about 2% of the soil N taken up in a cereal test crop was derived from the residue N (S. Laberge, personal communication).

3.1.7. NON-NITROGEN BENEFITS OF GRAIN LEGUMES FOR SUCCEEDING CROPS

The non-N benefits of grain legumes are numerous, but their significance is less well understood than the N related effects. The most well known are reduced root (take-all) and leaf diseases in subsequent cereal crops. Pea cultivation is associated with declines in pathogens causing diseases of wheat roots and leaves, reduced weed populations, reduced nematode infections, increased availability of P, potassium and sulphur, improved soil structure, releases of growth substances from legume residues, increased soil organic matter and increased microbial antagonists of soil-borne pathogens. Stevenson and van Kessel [32] studied the interactions between N and non-N benefits at the landscape scale. Using co-variance analysis to study the variability in the rotation (N and non-N) effects in terms of wheat grain yield, they showed significant co-variance between the rotational effect and increased available soil N (A_N value determination) derived from the pea phase, grassy weed

infestations and leaf diseases, which explained 9, 21 and 70%, respectively, of the variability in rotational effects among the subplots of the grids. Reduction in leaf diseases seemed to contribute the major part of the rotational effect in this study. Non-N benefits may have been indirectly responsible for greater N accumulation in the crop succeeding pea (N benefit); wheat accumulates more N when healthy (not infected with leaf pathogens), where there is less competition from weeds, as was also observed by Senaratne and Hardarson [26]. There remains a need to characterize in more detail the mechanisms behind the rotational effects of legumes, by determining the effects due to 'spared' N, residue N mineralized and non-N benefits, and the interactions between these factors.

3.1.8. CASE STUDIES

3.1.8.1. Case 1

This case study is based on data from Andersen et al. [7]. Biomass production, symbiotic N_2 fixation and inorganic N use were examined in dual- and tri-component annual intercrops.

3.1.8.1.1. Introduction

The aim of the study was to investigate N use dynamics in sole crops, dual- and tri-component intercrops of pea, barley and oilseed rape.

3.1.8.1.2. Methodology

Using a proportional replacement design, *Pisum sativum* L. (field pea), *Hordeum vulgare* L. (spring barley) and *Brassica napus* L. (oilseed rape) were grown as sole crops (SC), in dual-component intercrops and in a tri-component intercrop (IC), giving a total of seven crop treatments. The experiment was organized as a randomized split-plot design with four replicates (additional factors are not presented here). Each subplot (18 m^2) consisted of ten 12 m rows, spaced 12.5 cm apart. The crops were sown on 27 April. Sole-crop densities of 80 pea, 350 barley and 110 rape plants/m were aimed at. The two- and three-component crop mixtures consisted of one half and a third of the SC densities of each species, respectively. Pea, barley and oilseed rape seeds were sown consecutively in the same row; first the pea seeds were sown at a depth of 6 cm, then barley at 4 cm and lastly the rape seeds at a depth of 2 cm.

A ^{15}N microplot of ten rows of 2.7 m length was placed within each subplot. Each received the same amount of urea N as the subplots, but labelled with ^{15}N, i.e. 4 g N/m^2 at 2.5% ^{15}N atom excess. The ^{15}N enriched urea was dissolved in water and sprayed onto silica sand being stirred in a mixer. The treated sand was hand-spread as evenly as possible on the microplots, and immediately watered in with 2 L of tap water. Plots were fertilized on 10 May.

Crops were sampled five times, but only the final harvest data, 112 days after sowing, are shown here. Plant material was hand-collected from 1 m^2 at the final harvest. From the microplots, two rows of 0.5 m length were sampled. Harvested plant material was separated into component crops and individual biomass yields determined before and after drying at 80°C for 24 h. At the last harvest, pea pod walls and grain and rape seed were collected separately, and heads of barley were divided into grain and glume, before weighing. Nitrogen content and ^{15}N enrichment values were determined using a stable isotope ratio mass spectrometer coupled on-line to an elemental analyser (see Ref. [7] for more details).

TABLE I. at.% ^{15}N ENRICHMENT ABOVE GROUND BIOMASS, TOTAL N CONTENT, %Ndff, PER CENT RECOVERY OF FERTILIZER N (APPLIED AT 4 g N/m) AND %Ndfs IN SOLE (SC) AND INTERCROPS (IC) AT FINAL HARVEST

(the ^{15}N enrichment of the fertilizer was 2.5 at.% ^{15}N excess; averages of four replicates)

Crop	Component	at.% ^{15}N excess (SE)	Total N (g N/m^2)	%Ndff[a]	Fertilizer recovery[b] (%)	∑ recovery	%Ndfs
SC pea		0.17 (0.074)	18.7	7	32	32	31
SC barley		0.49 (0.031)	5.50	20	27	27	80
SC rape		0.42 (0.021)	7.49	17	32	32	83
IC pea/barley	Pea	0.07 (0.014)	4.22	3	3		13
	Barley	0.42 (0.038)	4.71	17	20	23	83
IC pea/rape	Pea	0.12 (0.015)	7.06	5	9		22
	Rape	0.46 (0.030)	6.21	19	28	37	81
IC barley/rape	Barley	0.42 (0.036)	4.54	17	19		83
	Rape	0.67 (0.094)	1.92	27	12	31	73
IC pea/barley/rape	Pea	0.07 (0.015)	4.18	3	3		11
	Barley	0.35 (0.029)	3.80	14	13		86
	Rape	0.64 (0.140)	2.53	26	18	34	74

[a] See Eq. (15).

[b] $\frac{\text{Amount of fertilizer in crop}}{\text{Amount of fertilizer applied}} \times 100$

[c] 100 – %Ndff – %Ndfa. (in principle, this pool includes any apparent N transfer).

3.1.8.1.3. Results

Fertilizer and soil-N uptake by the crops (Table I), and N_2 fixed by pea as well as the apparent transfer of N from pea to barley and rape, were determined (Table II). In the sole crops, barley took up less N from soil and fertilizer than did oilseed rape, but when the two species were grown in an IC, barley was the stronger competitor for soil and fertilizer N (Table I). Barley was also a stronger competitor for pea than was oilseed rape. Barley generally resulted in an overall low recovery of fertilizer N, and it is noteworthy that the pea/rape IC tended to recover more fertilizer N (Table I).

Clearly, intercropping increased the proportion of N in pea derived from fixation, but the amount of N per unit area was reduced compared to the SC, and in the pea/barley IC it was also lower than expected from the pea proportion in the IC (50%). This is due to competition for factors other than N (water, light, P, etc.), resulting in lower fixation (Table II). In the pea/rape IC, more inorganic N was available for pea, resulting in a lower %Ndfa. In the triple mixture, the amount of N fixed appeared to be close to the 'expected' level based on the composition of the IC (33% pea, or 11.7 × 0.333 = 3.9). Thus, as also discussed by Andersen et al. [7], it appears that oilseed rape has the ability to modify the competitive effects of barley.

The ^{15}N-enrichment (atom % excess) values of the crops in this experiment clearly highlight the weaknesses of the indirect ^{15}N approach for estimating N-transfer in field conditions (Table II). The enrichment of SC barley was not significantly different from that of barley in

the pea/barley IC (0.49±0.031 versus 0.42±0.038), yet the calculation showed an apparent %Ndft of 15% of the N in barley (Table II).

TABLE II. at.% ^{15}N EXCESS, PER CENT NITROGEN DERIVED FROM FIXATION, AMOUNT OF NITROGEN DERIVED FROM FIXATION, AND PER CENT NITROGEN IN THE NON-LEGUME INTERCROP COMPONENT FROM APPARENT NITROGEN TRANSFER

Crop	Component	at.% ^{15}N excess (SE)	%Ndfa[a]	N fixed (g/m^2)	%N transfer from pea[b]
SC pea		0.17 (0.074)	62	11.7	
SC barley		0.49 (0.031)			
SC rape		0.42 (0.021)			
IC pea/barley	Pea	0.07 (0.014)	85	3.6	
	Barley	0.42 (0.038)			15
IC pea/rape	Pea	0.12 (0.015)	73	5.1	
	Rape	0.46 (0.030)			–1
IC barley/rape	Barley	0.42 (0.036)			
	Rape	0.67 (0.094)			
IC pea/barley/rape	Pea	0.07 (0.015)	86	4.3	
	Barley	0.35 (0.029)			29
	Rape	0.64 (0.140)			–51

[a] See Eq. (10) in Ref. [8]; the reference crop value used in this study was the average of the enrichments in the SC barley and the SC oilseed rape (0.459 at.% ^{15}N excess).

[b] See Eq. (12).

The enrichment of rape was slightly higher when intercropped with pea, and higher still in the triple IC. In contrast, the barley enrichment was significantly lower in the triple IC than when sole cropped, indicating an apparent transfer of 29% of the N in barley in the IC. As already discussed, these estimates are associated with many potential errors and should be interpreted with caution. Competition between the species not directly associated with N may affect patterns of N uptake in the individual crops [7]. As an example, the competitive pressure of oilseed rape prolongs the active period of N uptake. Thus, if labelled N is temporarily immobilized during the early part of the growth season, then remineralized, and IC and SC crops have different patterns of uptake, differences in the crop enrichment values may result. Similarly, if IC barley is forced to take up unlabelled soil N from deeper soil layers as a result of competition [9], lower ^{15}N enrichment values will result in comparison with SC barley.

3.1.8.2. Case 2

This case study is based on unpublished data and on data generated in an experiment on N dynamics in legume based pasture systems [33].

3.1.8.2.1. Introduction

In some studies, it is necessary to determine how much N is derived from N_2 fixation by a preceding crop. This is possible using soil that was labelled in the organic fraction before establishing the N_2-fixing pre-crop. Jørgensen et al. [15] labelled field soil by immobilizing inorganic fertilizer N with sucrose and milled straw before establishing white clover,

perennial ryegrass or the clover/grass mixture. Two years after establishment, the pastures were ploughed in and barley was sown. It was assumed that any dilution of the soil N pool in plots containing clover compared to sole cropped ryegrass was due to N_2 fixation.

3.1.8.2.2. Labelling of soil with nitrogen-15 before grass pasture establishment

Nitrogen-15 was immobilized in the soil organic matter. Two weeks before sowing, an area of 250 × 160 cm in the middle of each plot was labelled with $(NH_4)_2SO_4$ enriched with ^{15}N at a rate of 10 kg N/ha. Sucrose and $(NH_4)_2SO_4$ at 30 at.% ^{15}N were dissolved in water and spread evenly on the soil surface by use of a syringe and a grid dividing the plot into subareas of 0.05 m^2. Finely milled barley straw (19 g/m^2) was then spread on the same subareas to obtain a C:N ratio of 22.5 in terms of the added sucrose, straw and $(NH_4)_2SO_4$. Subsequently the added materials were incorporated into the upper 15 cm of the soil by the use of a hand-held cultivator.

Mixtures and pure stands of perennial ryegrass (*Lolium perenne* L. cv. Sisu, Merlinda, Borvi and Tivoli) and white clover (*Trifolium repens* L. cv. Milkanova) were established in early May 1994 (Experiment A) and 1995 (Experiment B) by undersowing in a spring barley crop (*Hordeum vulgare* L. cv. Alexis) sown at a rate of 150 kg/ha. Seeding rates were as follows: white clover in pure stand 5 kg/ha, ryegrass in pure stand 25 kg/ha, and grass/clover mixtures 5 and 20 kg/ha for clover and ryegrass, respectively. Plots (440 × 160 cm) were laid out in a completely randomized block design with four replicates. After the 2 year forage crops, spring barley with undersown perennial ryegrass (same cultivars and seed rates as above) was established in the plots in 1997 and 1998, respectively, after ploughing in the forage crops.

The spring barley crop grown after forage was harvested in early August 1997 and 1998, and a cut was taken of the undersown ryegrass in late November and early December, respectively. Dried plant material was milled to a fine powder and analysed for total N and ^{15}N using an automated N analyser (Carlo Erba NA 1500) interfaced to a Finnigan MAT Delta continuous-flow isotope ratio mass spectrometer.

3.1.8.2.3. Results and discussion

White clover increased the succeeding spring barley yield by a factor of 2.4 and the total above ground N uptake by 7.2–11.4 g N/m^2 compared to grass as the preceding crop (Table III). Similarly, the grass/clover mixture increased the barley grain yield by a factor of 2.3 and the barley above ground N uptake by 6.0–8.6 g N/m^2. The residual N effect was partitioned into N derived from symbiotic N_2 fixation by the white clover pre-crop (non-labelled) and that from other sources (^{15}N labelled N from soil organic matter and clover residues) using Eq. (14) and the plot with the pre-crop grass as the control, assuming that the fixed N had diluted the ^{15}N enrichment of the plant available N pool. It was estimated that 27–30% of the N in the barley was derived from white clover fixed N after the pure clover, compared to 20–26% after the grass/clover mixture (Table IV). The dry matter production and N in above ground plant parts of ryegrass undersown to spring barley was more than doubled after clover and grass/clover compared to pure grass, when the crop was analysed in November/December (Table V). From 17 to 30% of the N in the perennial ryegrass catch crop was derived from N_2 fixation in the white clover of the pre-pre-crop (Table IV). Figure 1 shows the amount of N derived from fixation in barley. This N constituted only 40–50% of the difference in N uptake of barley–clover, based on the sole-crop grass pasture. Thus, the higher N uptake in crops following clover containing grass pastures compared to pure grass is only partly explained in terms of clover-fixed N. This may be more fully explained in terms

of the amount of labelled residue N possibly being much higher after the clover based pastures than after the grass pasture. Alternatively, the residual fixed N may have induced better barley growth with higher uptake of the labelled soil N and labelled pre-crop residue N.

TABLE III. DRY MATTER PRODUCTION, NITROGEN UPTAKE AND NITROGEN-15 ENRICHMENT IN SPRING BARLEY SUCCEEDING RYEGRASS, WHITE CLOVER OR GRASS/CLOVER, STUBBLE INCORPORATED IN SPRING

Experiment	Pre-crop	Dry matter yield		N content		Crop N (g/m^2)	Enrichment	
		Grain	Straw	Grain	Straw		Grain	Straw
		—— (g/m^2) ——		—— (%N) ——			(at.%^{15}N excess)	
A (1997)	Grass	272	261	1.1	0.74	5.00	0.117	0.109
	Clover	688	653	1.6	0.87	16.4	0.079	0.081
	Grass/Clover	627	584	1.4	0.81	13.6	0.086	0.082
B (1998)	Grass	256	277	1.4	0.88	5.90	0.111	0.127
	Clover	566	584	1.5	0.75	13.1	0.077	0.102
	Grass/Clover	587	543	1.5	0.80	11.9	0.089	0.100

TABLE IV. ESTIMATED PER CENT NITROGEN DERIVED FROM FIXATION BY WHITE CLOVER

Experiment	Pre-pre-crop	N from clover-fixed N		
		Barley grain	Barley straw	Grass cut Nov.–Dec.
		—————— (%) ——————		
A	Clover	33	26	30
	Grass/Clover	27	25	28
B	Clover	31	20	21
	Grass/Clover	20	21	17

TABLE V. DRY MATTER PRODUCTION, NITROGEN UPTAKE AND NITROGEN-15 ENRICHMENT OF RYEGRASS IN NOVEMBER/DECEMBER SUCCEEDING SPRING BARLEY AND PRE-PRE-CROPS OF RYEGRASS, WHITE CLOVER OR GRASS/WHITE CLOVER MIXTURE

Experiment	Pre-pre-crop	Grass		
		Dry matter	N	(at.% ^{15}N excess)
		——— (g/m^2) ———		
A	Grass	24	0.6	0.127
	Clover	65	2.1	0.089
	Grass/Clover	51	1.5	0.091
B	Grass	105	2.1	0.145
	Clover	195	4.1	0.115
	Grass/Clover	177	3.7	0.120

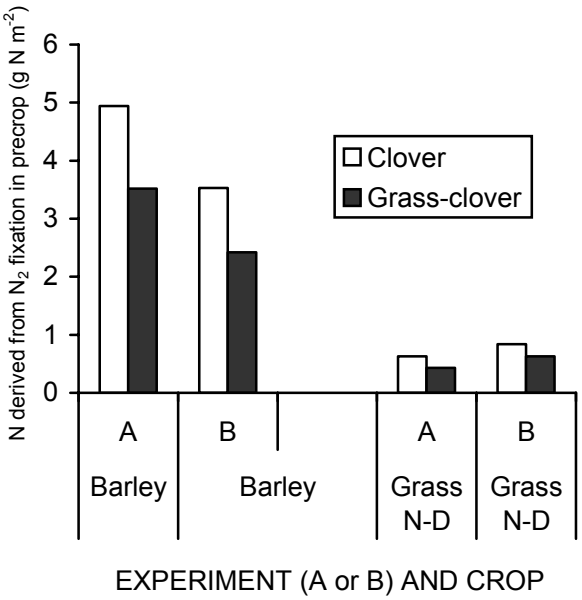

FIG. 1. Nitrogen (g/m^2) derived from nitrogen fixation in white clover and white clover/perennial ryegrass sward in the following barley crop and subsequent grass nitrogen-catch crop.

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3.2 USE OF NITROGEN-15 TRACERS TO QUANTIFY BIOLOGICAL NITROGEN FIXATION IN LEGUMINOUS CROPS

G. HARDARSON
FAO/IAEA Agriculture and Biotechnology Laboratory,
Agency's Laboratories Seibersdorf,
International Atomic Energy Agency,
Vienna

3.2.1. INTRODUCTION

Legumes are an important source of protein in the human diet. Their ability to assimilate atmospheric nitrogen in root nodules containing rhizobial bacteria means that they can yield well in soils deficient in mineral nitrogen, which makes them particularly important in agricultural systems in the developing world. In this session are described nitrogen-15 (^{15}N) methodologies used to quantify above and below ground nitrogen derived from the atmosphere.

Grain legumes are a particularly important source of protein. Although in terms of dry matter production, legumes (food legumes and leguminous oil-seeds) account for only 10% of the combined world yield of cereals and legumes, they constitute as much as 24% of the total protein yield.

One of the most important characteristics of legumes is their ability, in symbiosis with soil-borne rhizobial bacteria, to form nodules on their roots and to fix and utilize atmospheric nitrogen (N_2) for growth. Legumes with effective biological N_2 fixing capability can grow and yield well in N deficient soils without N fertilizer application. Therefore, they are particularly important in developing countries, due to the often high cost and/or restricted availability of N fertilizer.

For proper management and full realization of the benefits of this plant–microbe association, it is necessary to know how much N is fixed under various field conditions. Only after this is known can various factors be manipulated so as to increase the amount of N a crop derives from the atmosphere through biological N_2 fixation. A suitable method for accurately measuring the amount of N that crops derived from the atmosphere is therefore an important requirement in any programme aimed at maximizing biological N_2 fixation. The objective of this chapter is to illustrate the use of ^{15}N methodologies to quantify above and below ground N derived from the atmosphere through biological N_2 fixation.

3.2.2. METHODOLOGIES

Several methods are available to measure N_2 fixation [1], based on:

— increment in N yield and plant growth,
— N balance,
— acetylene reduction activity (ARA), and
— use of isotopes of N.

The selection of methodology will depend on the objective of the work. The various methods are compared in Table I. The dry matter yield, total N, ARA and xylem solute methods are

simple, rapid and relatively inexpensive [2]. They may be used in breeding programmes in which many analyses are necessary and precision is of secondary importance. When selecting plants for N_2 fixation related traits, sometimes it is necessary to evaluate them non-destructively for subsequent preservation of seed. For those that can be transplanted, ARA, xylem solute and fresh (instead of dry matter) yield methods may be used. Time integrated measurement of N_2 fixation and quantification of %Ndfa (percentage N derived from the atmosphere) and total Ndfa are particularly important for field measurements of various agronomic treatments and of breeding lines after the selection process. Total N, ^{15}N dilution, A-value and ^{15}N natural abundance methods are the most suitable for such a task, since only a few and relatively accurate measurements have to be made.

In this chapter, some of the isotopic methods will be illustrated in detail and reference is made to the other methods.

3.2.3. USE OF ISOTOPES TO MEASURE BIOLOGICAL NITROGEN FIXATION

In N isotope studies, the system under investigation is supplied with materials containing ^{15}N:^{14}N ratios measurably different from ^{15}N natural abundance. It is also essential that the N isotope ratio should again be measurably different from ^{15}N natural abundance at the time the system under investigation is sampled.

TABLE I. COMPARISON OF METHODS TO QUANTIFY SYMBIOTIC NITROGEN FIXATION [2]

Characteristic	DM[a]	Total N[b]	Nods[c]	ARA[d]	Xylem[e]	$^{15}N_2$[f]	ID[g]	AV[h]	NA[j]
Direct	–	–	–	–	–	+	–	–	–
No reference plant needed	–	–	+	+	+	+	–	–	–
Simple, rapid, inexpensive	++	+	+	++	+	–	–	–	–
Non-destructive	–(+)	–	+	+	(+)	–	–	–	–
Time integrated measure	+	+	–	–	–	–(+)	+	+	+
%Ndfa measured	–	+	–	–	–	–(+)	++	++	+
Measure of kg N/ha fixed	–	+	–	–	–	–	++	++	+
Small field variability	+	+	–	–	?	–	+	+	?

[a] Dry matter yield method.
[b] Total-N difference method.
[c] Nodule observation.
[d] Acetylene reduction assay.
[e] Xylem solute technique.
[f] Use of $^{15}N_2$ gas.
[g] ^{15}N isotope dilution.
[h] A-value method.
[j] ^{15}N natural abundance method.

3.2.3.1. Use of nitrogen-15 gas

The earliest application of $^{15}N_2$ in fixation studies was by Burris and Miller [3]. This method has been used to provide direct evidence for N_2 fixation; if fixation occurs, the ^{15}N concentration in plants exposed to $^{15}N_2$ is higher than the 0.3663% natural abundance. The extent to which ^{15}N is detected in the plant provides an estimate of the proportion of the plant's N that was derived from fixation, and is thus a direct method for quantifying N_2 fixed. The use of $^{15}N_2$ involves the enclosure of plants in chambers filled with the enriched gas [4]. The environment within the chamber is, however, likely to be different from that in a field situation. Also, it is difficult to confine plants in such chambers for long periods without affecting the growth conditions as compared to the field environment. Results obtained from such studies, therefore, tend to be instantaneous and subject to errors associated with extrapolating data from short term studies to a growth season, which involves diurnal and seasonal fluctuations [5].

3.2.3.2. Use of enriched fertilizer or substrates

The so-called isotope dilution method and other methods based on the same principle involve the growth of N_2-fixing (F) and non-fixing reference (NF) plants in soil fertilized with ^{15}N enriched inorganic or organic fertilizers. It relies on differential dilution in the plant of ^{15}N labelled fertilizer by soil and fixed N_2 [6–8]. It provides an integrated measurement of the amount of fixed N accumulated by a crop over the growth season.

The measurement of $\%^{15}N$ atom excess (a.e.) or per cent nitrogen derived from fertilizer (%Ndff) is necessary before N_2 fixation can be calculated. The following examples illustrate calculations of %Ndff for non-fixing and fixing crops.

Example 1

In a field experiment, 50 kg N/ha of 2.501% ^{15}N a.e. ammonium sulphate was applied to a cereal crop. At the end of the growth season, samples from the harvested material had 0.534% ^{15}N a.e. What was the %Ndff?

Calculation:

$$\%\text{Ndff} = \frac{\%^{15}\text{N atom excess}_{\text{plant}}}{\%^{15}\text{N atom excess}_{\text{fertilizer}}} \times 100 \quad (1)$$

$$\%\text{Ndff} = \frac{0.534}{2.501} \times 100 = 21\%$$

21% of the N in the plant was derived from fertilizer, and the remaining 79% was derived from soil.

A similar calculation can be made for legume crops, as shown in Example 2.

Example 2

20 kg N/ha of 5.231% ^{15}N a.e. was applied to a fixing (F) and a non-fixing (NF) crop. Samples from the harvested materials yielded 0.702 and 1.251% N a.e. for the F and NF crops, respectively. What were the %Ndff values for the two crops?

Calculation:

$$\%\text{Ndff} = \frac{\%^{15}\text{N atom excess}_{\text{plant}}}{\%^{15}\text{N atom excess}_{\text{fertilizer}}} \times 100$$

$$\%\text{Ndff}_{\text{F}} = \frac{0.702}{5.231} \times 100 = 13\%$$

$$\%\text{Ndff}_{\text{NF}} = \frac{1.251}{5.231} \times 100 = 24\%$$

In the NF crop (Example 1), the remaining 76% were derived from soil N. However, in the F crop the remaining 87% were derived from soil (%Ndfs) and atmosphere (%Ndfa, through biological N_2 fixation) as:

$$\%\text{Ndff}_{\text{F}} + \%\text{Ndfs}_{\text{F}} + \%\text{Ndfa} = 100$$

The question, therefore, remains: what were the proportions derived from air and soil in the F crop? To calculate the relative proportions derived from these two sources, it is necessary to assume that both, non-fixing and fixing crops, take up N from soil and fertilizer in the same ratio, i.e.

$$\frac{\%\text{Ndff}_{\text{NF}}}{\%\text{Ndfs}_{\text{NF}}} = \frac{\%\text{Ndff}_{\text{F}}}{\%\text{Ndfs}_{\text{F}}} \qquad (2)$$

Using this equation, the calculation of Example 2 can be continued as illustrated in the following table:

Component	$\%^{15}$N a.e.	%Ndff	%Ndfs	$\frac{\%\text{Ndff}}{\%\text{Ndfs}}$	%Ndfa
NF	1.251	23.9	76.1	0.314[a]	0
F	0.702	13.4	42.7[b]	0.314[a]	43.9[c]
Fertilizer	5.231				

According to the above assumption:

[a] $$\frac{\%\text{Ndff}_{\text{NF}}}{\%\text{Ndfs}_{\text{NF}}} = \frac{23.9}{76.1} = 0.314 = \frac{\%\text{Ndff}_{\text{F}}}{\%\text{Ndfs}_{\text{F}}}$$

Thus

[b] $$\%\text{Ndfs}_{\text{F}} = \frac{\%\text{Ndff}_{\text{F}}}{0.314} = \frac{13.4}{0.314} = 42.7$$

and

[c] $$\%\text{Ndfa} = 100 - \%\text{Ndff}_{\text{F}} - \%\text{Ndfs}_{\text{F}}$$

$$\%\text{Ndfa} = 100 - 13.4 - 42.7 = 43.9$$

In this way, the proportions of N from all available sources have been quantified, i.e. for the non-fixing crop (NF)

%Ndff = 23.9
%Ndfs = 76.1

and for the fixing crop (F)

%Ndff = 13.4
%Ndfs = 42.7
%Ndfa = 43.9

The methodology is certainly not precise enough to measure decimal points meaningfully, so one should report the %Ndfa as 44%. But what is the accuracy of the ^{15}N dilution method? That question is addressed below.

There are five main variations in the use of ^{15}N labelled substrates:

— the ^{15}N isotope dilution method (ID), which was partly illustrated above [6, 8],
— the A-value method (AV) [7],
— the single-treatment method (ST) [9],
— the yield independent method [10] and
— the natural abundance method [11, 12].

3.2.3.2.1. The isotope dilution method

In this case, both fixing and reference plants are grown on soil to which the same amount of fertilizer, having the same ^{15}N enrichment, has been applied, as illustrated in Example 2. Thus, in the absence of a supply of N other than soil and ^{15}N labelled fertilizer, a fixing plant and a non-fixing reference plant will contain the same ratio of ^{15}N:^{14}N, since they are taking N of similar $^{15}N/^{14}N$ composition, although not necessarily the same total quantity of N. In both cases, the ^{15}N:^{14}N ratio within the plant is lowered by the N absorbed from the unlabelled soil. However, in the presence of N_2, the fixing plant further lowers the ratio of ^{15}N:^{14}N due to incorporation of N from unlabelled air, whereas this does not occur in the non-fixing plant. The extent to which the ^{15}N:^{14}N ratio in the fixing crop is decreased, relative to the non-fixing plant, is, therefore, an indication of N_2 fixing ability, and can be used to estimate the amount of N fixed under field conditions.

The determination of the amount of N fixed using this approach is depicted in Fig. 1 with a theoretical example. By using ^{15}N labelled fertilizer, 50% of the N in the NF reference crop was derived from the applied fertilizer. Since only two sources of N are available to this crop,

$$\%Ndff_{NF} + \%Ndfs_{NF} = 100 \quad (3)$$

or

$$a + b = 100$$

It follows from Eq. (3) that the other half, or 50%, of the N in the plant came from soil. This then establishes that the ratio of soil to fertilizer N available to the non-fixing plant was 1:1 in this example.

For the legume in Fig. 1, a third source of N is available, i.e. N_2 from the atmosphere. The total N in the plant can, therefore, be represented by the following equation:

$$\%Ndff_F + \%Ndfs_F + \%Ndfa = 100 \quad (4)$$

or

$$c + d + e = 100$$

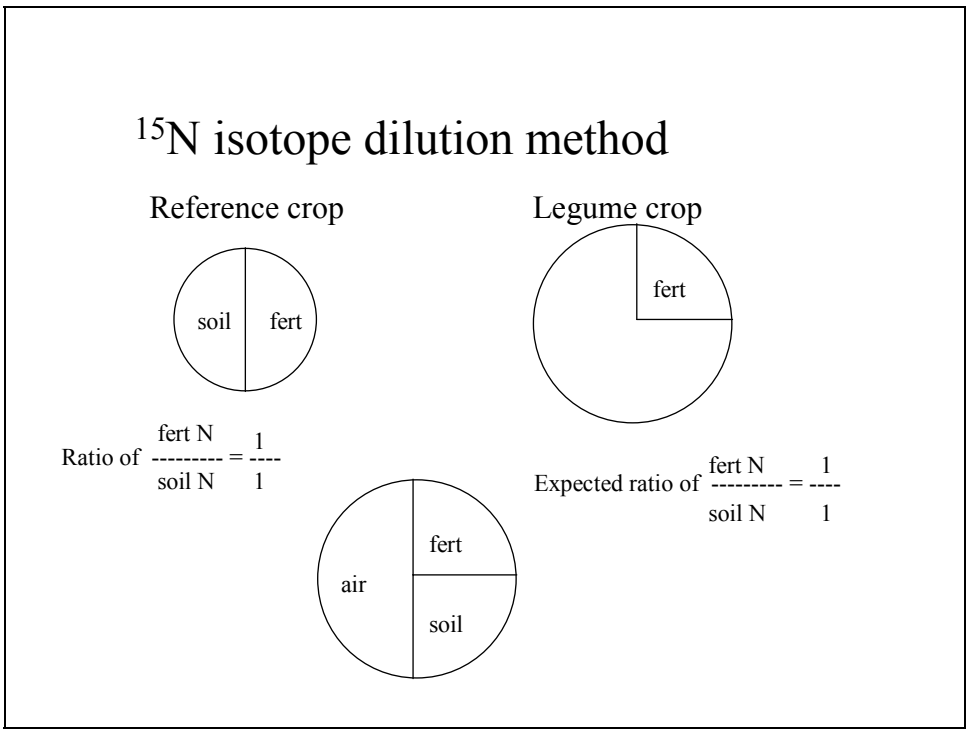

FIG. 1. Simple example of how the isotope dilution technique is used to measure nitrogen fixation by grain legumes.

The non-fixing reference crop took up N from soil and fertilizer in the ratio 1:1, and it is assumed, as shown in Eq. (2), that the same occurred in the fixing crop, i.e.

$$\frac{a}{b} = \frac{c}{d}$$

In the example, %Ndff in the fixing crop was 25%. Therefore, according to Eq. (2), the %Ndfs in the fixing crop was also 25%. The rest of the N taken up (50%) was derived from the atmosphere, since, according to Eq. (4):

$$\% \text{ Ndfa} = 100 - (\%\text{Ndff}_F + \%\text{Ndfs}_F)$$

%Ndfa, as quantified by the ^{15}N dilution method, is usually calculated by the following equation:

$$\%\text{Ndfa} = (1 - \frac{\%\text{Ndff}_F}{\%\text{Ndff}_{NF}}) \times 100 \quad (5)$$

Derivation of Eq. (5)

Equation 5 is derived from Eqs (2)–(4) as follows:

From Eq. (4),

$$e = 100 - c - d \quad (6)$$

and from Eq. (2)

$$d = \frac{c \times d}{a} \quad (7)$$where, according to Eq. (3),

$$b = 100 - a$$

From Eqs (3) and (7),

$$d = \frac{c}{a} \times (100 - a)$$

or

$$d = \frac{100 \times c}{a} - c \tag{8}$$

From Eqs (6) and (8),

$$e = 100 - c - (\frac{100 \times c}{a} - c)$$

or

$$e = (1 - \frac{c}{a}) \times 100 \tag{9}$$

or

$$\%Ndfa = (1 - \frac{\%Ndff_F}{\%Ndff_{NF}}) \times 100$$

Equation 9 can be written as:

$$\%Ndfa = (1 - \frac{\%^{15}Na.e._F}{\%^{15}Na.e._{NF}}) \times 100 \tag{10}$$

since

$$\%Ndff = \frac{\%^{15}N\ atom\ \ excess_{sample}}{\%^{15}N\ atom\ \ excess_{fertilizer}} \times 100 \tag{11}$$

The amount of N_2 fixed can be calculated according to:

$$\text{Amount of } N_2 \text{ fixed} = \frac{\%Ndfa \times \text{total } N_F}{100} \tag{12}$$

The uses of Eqs (5), (10) and (12) are illustrated in Table II.

TABLE II. FIELD-GROWN NODULATING (F) SOYBEAN AT THE SEIBERSDORF LABORATORY

(20 kg N/ha of ^{15}N fertilizer was applied to fixing and non-fixing crops; data for one of five replicates are shown)

Plant part	DM yield[a] (kg/ha)	N[b] (%)	N yield[d] (kg/ha)	^{15}N a.e.[c] (%)	Ndff[e] (%)	N fert. yield[f] (kg/ha)	Ndfa[g] (%)	Fixed N[h] (kg/ha)
Stems	4,478	0.63	28.2	0.152	3.16	0.89		
Leaves	2,743	1.9	52.1	0.158	3.28	1.71		
Pods	1,867	2.6	48.2	0.132	2.74	1.32		
Total			129		3.05[J]	3.92	26	33

Values needed for the calculation:

%^{15}N a.e. of fertilizer: 4.81
%$Ndff_{NF}$: 4.14 (calculated by the same method as %$Ndff_F$)

Measured values in Table II:

[a] Dry matter yield of plant parts (kg/ha).
[b] %N of each plant part in dry matter yield.
[c] %^{15}N a.e. of each plant part in dry matter yield and of fertilizer applied.

Calculated values in Table II:

$$^{d}\ \text{N yield (kg/ha) of each plant part} = \frac{\text{DM of each plant part} \times \%\text{N}}{100}$$

$$^{e}\ \%\text{Ndff} = \frac{\%^{15}\text{N atom excess}_{\text{sample}}}{\%^{15}\text{N atom excess}_{\text{fertilizer}}} \times 100$$

$$^{f}\text{N fertilizer yield (kg/ha)} = \frac{\text{N yield (kg/ha)} \times \%\text{Ndff}}{100}$$

$$^{g}\ \%\text{Ndff(weighed average)} = \frac{\text{Total N} - \text{fert. yield}}{\text{Total N yield}} \times 100$$

$$^{h}\ \%\text{Ndfa} = (1 - \frac{\%\text{Ndff}_{\text{F}}}{\%\text{Ndff}_{\text{NF}}}) \times 100$$

or

$$\%\text{Ndfa} = (1 - \frac{\%^{15}\text{N a.e.}_{\text{F}}}{\%^{15}\text{N a.e.}_{\text{NF}}}) \times 100$$

$$^{j}\ \text{N}_2\ \text{fixed (kg/ha)} = \frac{\%\text{Ndfa} \times \text{total N}_{\text{F}}}{100}$$

Example 2 (cont.)

It is possible to use Eqs (9) or (10) to calculate %Ndfa in the above example:

$$\%\text{Ndfa} = (1 - \frac{\%\text{Ndff}_{\text{F}}}{\%\text{Ndff}_{\text{NF}}}) \times 100$$

$$\%\text{Ndfa} = (1 - \frac{13.4}{23.9}) \times 100 = 43.9\%$$

or

$$\%\text{Ndfa} = (1 - \frac{\%^{15}\text{N a.e.}_{F}}{\%^{15}\text{N a.e.}_{NF}}) \times 100$$

$$\%\text{Ndfa} = (1 - \frac{0.702}{0.251}) \times 100 = 43.9\%$$

Assumption inherent in the isotope dilution methodology

The assumption made in Eq. (2) is the only one inherent in the ^{15}N dilution methodology, i.e. that both fixing and non-fixing crops take up N from soil and fertilizer in the same ratio. For this to be true, the fixing and the non-fixing (reference) crops have to match and the following conditions have to be met [13]:

— Either fertilizer distribution is even with depth, or the legume and reference crops have spatially similar nutrient uptake profiles, i.e. the root systems should be similar.
— The contribution of seed N should be negligible, which is not always true, especially if the plants are harvested early in the growth season.
— It is implicit in the calculation that the enrichment of plant available soil N remains constant with time or that the legume and control have similar N uptake patterns. In practice, when fertilizer N is added as a single application, the enrichment of plant available soil N declines with time, and this decline can vary between the legume and the control plant. Depending on whether the control takes up soil N faster or more slowly than the legume, the calculated N_2 fixation rate will be higher or lower than the true value [14]. Errors due to making this assumption may be reduced by use of slow-release N fertilizer and by choice of a control plant that closely parallels the legume in its N uptake [13].

Accuracy of measurements of nitrogen fixation

The accuracy and precision of the isotope dilution method depend to a great extent on selecting a suitable NF reference crop. Selecting the appropriate reference plant is crucial; it is essential to observe the following points:

— The reference crop does not fix N_2. If necessary, this can be checked quickly using the acetylene reduction assay.
— The rooting depths of both reference and fixing crops are similar, or both crops should derive their entire N from the same zone.
— The N_2 fixing and reference crops have similar growth or physiological stages, and mature at about the same time.
— The N_2 fixing and reference crops should be planted and harvested at the same time.
— Both crops should be affected in a similar fashion by changes in environmental conditions, such as temperature and water, during the growth period.

The following NF reference crops have been used for estimating N_2 fixed in grain legumes:

— A non-legume, non-fixing plant.
— A non-nodulating legume plant.
— An uninoculated legume plant in soils devoid of compatible strains of rhizobia.

Even if these conditions apply, there is no guarantee that the measurement of N_2 fixation will be correct. Only through several experiments can one gain confidence in the system under study and an understanding of the accuracy of the measurements.

Methods based on dilution in the plant of ^{15}N labelled fertilizer by N derived from the atmosphere and from the soil seem to offer a potentially accurate means of quantifying symbiotic N_2 fixation. Variations are, however, often found, depending on the non-fixing reference crop [15]. This has been found to be mainly due to differences in N uptake pattern of the legume and control combinations, together with a decrease in the ^{15}N:^{14}N ratio of the substrate with time [14].

It has been observed at the Seibersdorf Laboratory that the ^{15}N methodology seems to be particularly accurate when a large proportion of the N in the fixing plant is derived from the atmosphere [16, 17]. This prompted us to model the percentage N derived from the atmosphere in relation to ^{15}N enrichment in the fixing and non-fixing standard crops, and to investigate where major errors in estimates of N_2 fixation can be expected.

As shown previously, %Ndfa ("e") is calculated using the isotope dilution method using Eqs (9) or (10), i.e.

$$e = (1 - \frac{c}{a}) \times 100$$

where *c* and *a* are either $\%^{15}N$ a.e. or %Ndff of the fixing and non-fixing crops, respectively.

Using this equation, modelled curves for various *c* and *a* values of both fixing and non-fixing crops are shown in Fig. 2. When c is 1%, e increases very rapidly up to 80% with increased values of *a*. At higher *c* values, differences in *a* do not affect *e* to the same extent. A 10% coefficient of variation of *a* (%Ndff or % N atom excess of the reference crop) produces much larger variation in *e* (%Ndfa) when it is small (Fig. 2 and Table III).

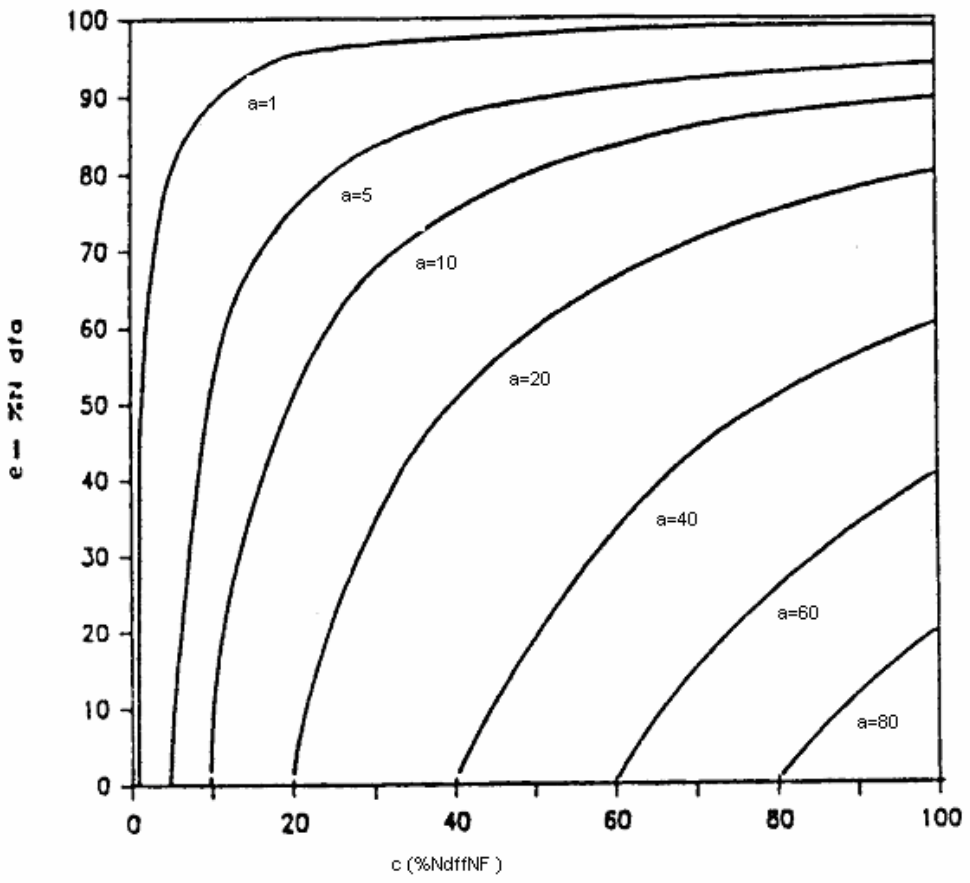

FIG. 2. Modelled curves for e (%Ndfa) at various c (%Ndff$_{NF}$) and a (% Ndff$_{F}$) values.

TABLE III. CALCULATED VALUES FOR *e* (%Ndfa) FROM VARIOUS VALUES OF *c* (%^{15}N A.E. OR %Ndff OF FIXING CROP) WHEN *a* (%^{15}N A.E. OR %Ndff OF REFERENCE CROP) HAS A 10% COEFFICIENT OF VARIATION

Example	a	c	e	Range (%)
1	20 ± 2	18	0–18	18
2	20 ± 2	12	33–45	12
3	20 ± 2	8	55–64	9
4	20 ± 2	4	78–82	4
5	20 ± 2	2	89–91	2

It is clear from the above modelling that methods based on dilution in the plant of ^{15}N labelled fertilizer by N derived from the atmosphere are potentially accurate for quantification of N_2 fixation when large proportions (>70%) of the N in the fixing crop are derived from fixation. However, at lower N_2 fixation levels (<30%), the methodology is much less accurate; under such conditions, the selection of the reference crop and the stability of the ^{15}N:^{14}N ratio of the substrate are particularly important.

3.2.3.2.1.1. Greenhouse experiments

The ^{15}N dilution method can be used to quantify biological N_2 fixation in pot experiments under greenhouse conditions. In this case the soil or substrate must be uniformly labelled with the ^{15}N tracer.

Pots/soil: To measure N_2 fixation in, for example, soybean inoculated with ten strains of *Bradyrhizobium japonicum* under greenhouse conditions, one would need at least 48 pots, i.e. four for each inoculation treatment, four for the uninoculated control and four for the non-fixing reference plants. Using pots that hold 10 kg of soil, 480 kg of soil would be needed. If the pots have drainage holes, saucers will prevent loss of ^{15}N through excessive watering.

^{15}N tracer: The equivalent of 10 kg N/ha of 10% ^{15}N a.e. as ammonium sulphate or urea would be sufficient to evaluate N_2 fixation in such an experiment. To calculate how much N is needed, 1 ha of soil is assumed to weigh 2 million kg.

For 2 million kg of soil, 10 kg of N would be applied; therefore, for 480 kg of soil, the equivalent rate is 2.4 g of N.

With ammonium sulphate, which is approximately 21% N, 11.43 g will be added to 480 kg of soil. The 11.43 g of ammonium sulphate is dissolved in water to make 2400 mL, added in 50 mL aliquots to the soil of each pot, and mixed thoroughly.

Treatments/sowing: The soybean seed has to be inoculated with each of the strains of rhizobia, and the pots sown. Care is needed not to contaminate the uninoculated treatment. The non-nodulating soybean (reference crop) should not be inoculated. Four seeds are sown and thinned to two seedlings per pot. The treatments should be randomized and the pots watered.

Harvest/analyses: After the growth period, the plants are harvested and analysed.

*3.2.*3.2.1.2. Field experiments

A similar experiment may be done under field conditions. Usually the treatments would be fewer than in the greenhouse experiment, e.g. one might test five of the best rhizobial strains tested previously under greenhouse conditions.

Plots: After preparation of the experimental area, the plots have to be measured and laid out. It is best to mark the borders of the plots with string. To be able to measure N_2 fixation of a legume crop, plots of the legume and others with a non-fixing reference crop are required. One reference plot in each replication is sufficient if strains of rhizobia are being tested. However, if phosphorus (P) application or time of harvesting are being studied, the reference crop has to be treated in the same way as the fixing crop. In this case, one plot of reference crop is needed for each plot of fixing crop. Isotope plots of 1–3 m^2 containing at least 20 harvestable plants per plot are usually sufficient.

^{15}N application: Approximately 0.1 g N/m^2 , i.e. 20 kg N/ha of 5% ^{15}N a.e. or 100 kg N/ha of 1% ^{15}N a.e. is usually enough for detection in the plants. Urea, ammonium nitrate or

ammonium sulphate fertilizer can be applied in solid or liquid form (fertilizer dissolved in at least 500 mL water/m^2). The fertilizer should not be applied when the soil temperature is very high, or direct volatilization of ammonia will occur.

Harvest/analyses: At the time of harvest, the plots of the reference crop should be harvested at the same time as those of the fixing crops. Pods and straw are usually very different in %N and $\%^{15}N$ a.e. values, therefore it is not possible to obtain directly representative subsamples from mixtures of those plant parts. The plants should, therefore, be separated into pods and straw, which are weighed and subsampled after chopping into small fragments. Material with lower $\%^{15}N$ a.e. values should be chopped first, to minimize contamination effects. In any case, the forage chopper, if used, must be cleaned thoroughly between treatments. Subsamples should be ground after drying at 70°C. These samples can then be analysed for total N by the Kjeldahl procedure. The ^{15}N:^{14}N ratio can be analysed by either emission or mass spectrometry.

Calculation: After the analyses of $\%^{15}N$ abundance in the plant and fertilizer samples, $\%^{15}N$ a.e. has to be calculated by subtracting the $\%^{15}N$ natural abundance (0.3663%) from the $\%^{15}N$ abundance in the sample. The $\%^{15}N$ a.e. values are used for all the following calculations; % N derived from fertilizer (%Ndff) is the first derived value, as shown below.

3.2.3.2.2. The A-value method

Often, it is necessary to apply different doses of N to fixing and non-fixing plants. As high levels of inorganic N can depress N_2 fixation, it is necessary to apply low amounts of labelled N fertilizer to the fixing crop in order to estimate N_2 fixed. However, such amounts may be too low to support adequate growth of the reference plants, especially in soils of low fertility. For these reasons it is practical to give a reasonable dose of ^{15}N labelled fertilizer (40–80 kg N/ha) to the reference crop, while the fixing crop receives a low quantity (5–20 kg N/ha) [7]. It is recommended that the isotope dilution method be used whenever possible and the A-value method employed when the reference crop would be unable to grow well due to lack of N in the soil.

When different fertilizer rates are applied to the F and NF crops, *n* is the relative amount of fertilizer applied, i.e. the amount of fertilizer applied to the F crop divided by the amount applied to the NF crop.

The assumption (Eq. (2)) which was previously presented for the isotope dilution method is also used for the A-value methodology, but *n* $\%Ndff_{NF,}$ which is the estimated $\%Ndff_{NF}$ at the rate of the F crop, has to be calculated using the following equation:

$$\frac{n \times \%Ndff_{NF}}{\%Ndfs_{NF}} = \frac{\%Ndff_{F}}{\%Ndfs_{F}} \quad (13)$$

Example 3

In a field experiment, two rates of ^{15}N labelled ammonium sulphate were applied to the F and NF crops, i.e. 20 kg/ha of 5.6% ^{15}N a.e. to the F crop and 60 kg N/ha of 2.5% ^{15}N a.e. to the NF crop. What was the %Ndfa for the F crop?

Calculation:

Crop	Fert. rate (kg N/ha)	%^{15}N a.e. (fertilizer)	%^{15}N a.e. (plant)	%Ndff	%Ndfs	%Ndff / %Ndfs	%Ndfa
NF	60	2.50	0.40	16	84	0.063[a]	
F	20	5.60	0.08	1.4	22.1[b]	0.063[a]	76.5[c]

[a] $\frac{n \times \%Ndff_{NF}}{\%Ndfs_{NF}} = \frac{0.33 \times 16}{84} = 0.063 = \frac{\%Ndff_F}{\%Ndfs_F}$

[b] $\%Ndfs_F = \frac{1.4}{0.063} = 22.1\%$

[c] %Ndfa = 100 – $\%Ndff_F$ – $\%Ndfs_F$

%Ndfa = 100 – 1.4 – 22.1 = 76.5%

Example 3 can also be calculated using the following equation:

$$\%\text{Ndfa} = 100\left(1 - \frac{\%\text{Ndff}_F}{n \times \%\text{Ndff}_{NF}}\right) + \%\text{Ndff}_F\left(\frac{1}{n} - 1\right) \quad (14)$$

$$\%\text{Ndfa} = 100\left(1 - \frac{1.4}{0.33 \times 16}\right) + 1.4\left(\frac{1}{0.33} - 1\right) = 76.5\%$$

Equation 5 of the ^{15}N dilution method is a particular case of Eq. (14) when *n* is equal to 1.

Derivation of Eq. (14) is shown in Ref. [18].

TABLE IV. DATA RECORDED FOR FABA BEAN (F) AND BARLEY (NF) AT THE SEIBERSDORF LABORATORY

(20 kg N/ha of ^{15}N labelled fertilizer were applied to the bean and 100 kg N/ha to the barley; one of five replicates is shown)

Crop	N fertilizer rate (kg N/ha)	Total N yield[a] (kg/ha)	%Ndff[b]	%Ndfa[c]	Fixed N[d] (kg N/ha)
F	20	151.7	0.877	79	120
NF	100		18.17		

Measured values (not shown):
Dry matter yield of plant parts (kg/ha)
%N of each plant part
%^{15}N a.e. of each plant part and of the fertilizer applied

Values needed for the calculation:
%^{15}N a.e. of fertilizer, F: 5.64
%^{15}N a.e. of fertilizer, NF: 1.00

Calculated values in Table IV :

[a] $$\text{N yield (kg/ha) of each plant part} = \frac{\text{DM of each plant part} \times \%\text{N}}{100}$$

[b] $$\%\text{Ndff} = \frac{\%^{15}\text{N atom excess}_{\text{sample}}}{\%^{15}\text{N atom excess}_{\text{fertilizer}}} \times 100$$

[c] $$\%\text{Ndfa} = 100\left(1 - \frac{\%\text{Ndff}_{\text{F}}}{n \times \%\text{Ndff}_{\text{NF}}}\right) + \%\text{Ndff}_{\text{F}}\left(\frac{1}{n} - 1\right)$$

[d] $$\text{N}_2 \text{ fixed (kg/ha)} = \frac{\%\text{Ndfa} \times \text{total N}_{\text{F}}}{100}$$

This method was originally presented using the A-value concept of Fried and Broeshart [7].

3.2.3.2.3. Single-treatment method

The third variation of the isotope dilution method was introduced by Fried and Broeshart [7]. As this method is not in common use, it will not be explained here.

3.2.3.2.4. Yield independent model

This method is based on measurement of the temporal change in the isotopic composition of the pool of available soil N, which is being exploited by the legume roots. The integrated pool enrichment is used as the reference criterion, instead of the N taken up by a non-fixing plant as in the isotope dilution method. The method and the equations used have been explained in detail by Chalk et al. [19] and Chalk and Ladha [10].

3.2.3.2.5. Natura abundance method

As a result of isotope discrimination effects occurring during formation, most soils have slightly higher ^{15}N abundance values than does the atmosphere. As a result, N_2-fixing plants have been found to have lower levels of ^{15}N enrichment than do non-fixing plants, which has been used as evidence for, and as a measure of, N_2 fixation [11, 12].

The level of natural ^{15}N abundance is often expressed in terms of the more sensitive unit; δ^{15}N (parts per thousand, ‰) is often used [20]:

$$\delta\,^{15}‰ = \left(\frac{^{15}\text{N}/^{14}\text{N}_{\text{sample}}}{^{15}\text{N}/^{14}\text{N}_{\text{standard}}} - 1\right) \times 1000$$

If N_2-fixing plants are grown in soil that has a higher δ^{15}N value than the atmosphere, then %Ndfa can be calculated according to the following equation:

$$\%\text{Ndfa} = \left(1 - \frac{\delta^{15}\text{N‰}_{\text{F}} - \delta^{15}\text{N‰}_{\text{air}}}{\delta^{15}\text{N‰}_{\text{NF}} - \delta^{15}\text{N‰}_{\text{air}}}\right) \times 100$$

The main advantage of this method is that no tracer has to be applied. The method is, therefore, particularly useful for natural ecosystems, e.g. with trees, for which it is difficult to label the substrate. However, the main limitations are the rather small differences in ^{15}N abundance being measured and the high variability of ^{15}N abundance in soils.

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3.3. NITROGEN BALANCE IN CROPPING SYSTEMS THAT INCLUDE LEGUMES

M.B. PEOPLES
CSIRO Plant Industry,
Canberra,
Australia

3.3.1. BACKGROUND

This session explores the data required to quantify a system's nitrogen balance using crop legumes as an example, with particular emphasis on the methodologies that might be used to quantify the below ground contributions of nitrogen associated with roots and root nodules.

There are a number of pathways by which nitrogen (N) inputs via biological N_2 fixation (BNF) can improve the N fertility of soils for the benefit of other crops (Table I). However, the actual contribution of a legume–rhizobia symbiosis to the N balance of an ecosystem will primarily be determined by the difference between the total input of fixed N and the amount of N removed in agricultural produce or plant residues. The principles will be the same, regardless of whether the legumes are annuals or perennials grown for grain, as a green manure, or for forage, although, in the case of grazed forage or pasture legumes, estimates of N losses from urine patches should be included in the calculations (e.g., Ref. [1]).

3.3.2. CALCULATING A NITROGEN BALANCE

The N present in a legume crop (NLeg) can be calculated from the amount of dry matter accumulated during growth (LegDM) and the N content (%N) of that dry matter. The measured NLeg can be separated into that proportion of the plant N derived from atmospheric N_2 (Ndfa) and that assimilated from soil mineral N using one or more of the techniques described in Ref. [2] and reviewed by Peoples et al. [3].

TABLE I. PATHWAYS OF NITROGEN TRANSFER FROM N_2-FIXING LEGUMES TO SOIL ORGANIC AND INORGANIC POOLS, OR TO OTHER PLANTS

(modified from Ref. [4])

Pathway		Rate of transfer	Likely importance as an N source
Above ground	Decomposition and mineralization of senesced leaves and stover	Slow	Major
	Consumption of foliage by grazing animals and return via excreta	Slow (manure) Rapid (urine)	Major
	Foliar leachates	Rapid	Minor
	Volatile losses of ammonia	Rapid	Minor
Below ground	Mineralization of root and nodule N	Slow	Major
	Rhizodeposition (N exudation from roots)	Rapid	Minor/Major (?)
	Transfer via mycorrhizal hyphae	Rapid	Minor

TABLE II. RANGES OF EXPERIMENTAL ESTIMATES OF THE PROPORTION OF PLANT N DERIVED FROM ATMOSPHERIC NITROGEN (Ndfa) AND AMOUNTS OF SHOOT NITROGEN FIXED (Nfix) BY IMPORTANT LEGUME CROPS

(collated from Refs [4, 5–7])

Species	Ndfa (%)	Nfix (kg N/ha)
Warm-season legumes		
Soybean (*Glycine max*)	0–95	0–450
Common bean (*Phaseolus vulgaris*)	0–73	0–165
Groundnut (*Arachis hypogaea*)	16–92	21–206
Cowpea (*Vigna unguiculata*)	32–89	9–201
Pigeon pea (*Cajanus cajan*)	0–100	0–235
Cool-season legumes		
Chickpea (*Cicer arietinum*)	0–82	0–141
Field pea (*Pisum sativum*)	5–95	4–244
Lentil (*Lens culinaris*)	28–87	5–192
Faba bean (*Vicia faba*)	19–97	12–330
Lupin (*Lupinus angustifolius*)	20–97	19–327

Estimates of Ndfa and amounts of N_2 fixed (Nfix) by important tropical and cool-season crop legumes are presented in Table II. Although experimental treatments and environmental or nutritional variables have generated a large range of Ndfa values (0–100%), it appears that the potential for BNF by most crop legume species is between 150 and 300 kg N/ha (Table II).

By the end of the growth season, the N in a crop legume is partitioned into the seed (NLs) and various vegetative parts that remain as crop residues after the seed is harvested. That proportion of the total crop N that is present in the seed is described by the harvest index for N (NHI).

For crop legumes to play an important role in maintaining the soil-N fertility for other crops in a rotation, they need to fix more N than is removed with the harvested seed. The net contribution of N_2 fixation to the N balance of a soil following legume cropping can be calculated as (after Ref. [8]):

$$\text{Net N balance} = (\text{Nfix} - \text{NLs}) \quad (1)$$

where

$$\text{Nfix} = \frac{\text{Ndfa} \times \text{NLeg}}{100} \quad (2)$$

$$\text{NLs} = \frac{\text{NHI} \times \text{NLeg}}{100} \quad (3)$$

$$\text{NHI} = \frac{\text{NLs}}{\text{NLeg}} \times 100 \quad (4)$$

$$\text{NLeg} = \frac{\text{LegDM} \times \%\text{N}}{100} \quad (5)$$

Given Eqs (2) and (3), Eq. (1) can also be written as in Ref. [9]:

$$\text{Net N balance} = \frac{(\text{Ndfa} \times \text{NLeg}) - (\text{NHI} \times \text{NLeg})}{100} = \frac{\text{NLeg}(\text{Ndfa} - \text{NHI})}{100} \quad (6)$$

With protein levels of between 20 and 40%, legume seeds have a high demand for N. For example, up to 60 kg N/ha will be removed with every metric tonne (t) of grain harvested for a crop such as soybean. Therefore, given a global average yield for soybean of about 2 t/ha, 120 kg N/ha will be removed in the grain. Concomitantly, at least 120 kg N/ha would have to be fixed by soybean during growth for the soil to receive any net benefit from BNF.

Reported estimates of the N balance calculated using Eq. (1) following various temperate and tropical legume crops range from around –150 kg N/ha (i.e. the amount of N_2 fixed was 150 kg N/ha less than the N removed in the harvested seed) to +135 kg N/ha [4, 8, 9]. These values represent measures of the potential net benefits from BNF and assume that all residual vegetative N remains in the field after grain harvest. The actual input of fixed N will be less than this potential if crop residues are removed from the field or grazed by animals.

It is clear from Eq. (6) and by examining the data presented in Table III that the N balance will be positive if Ndfa > NHI, and will be negative when Ndfa < NHI. Thus, crop legumes with high biomass N, low NHI and high reliance upon N_2 fixation for growth have the greatest potential to have a net positive contribution of fixed N to soil [9].

Table III also indicates that NHI is not necessarily a constant. Reported measures of NHI range from 20 to 90% for various legume species [4, 8, 9]. The nitrogen harvest index can be influenced by any factor that affects either seed yield or total biomass production. This includes genetic (legume species, variety), environmental (geographic location, growth season, annual differences in rainfall and/or temperature) and management variables (rotations, irrigated or rainfed, nutrient availability, foliage and root disease, insect pests). Consequently, NHI should routinely be determined in each experiment.

Several potential sources of error need to be considered when calculating the N balance of a legume based agroecosystem. These include the timing of plant sampling, sampling protocols used to quantify legume biomass, and the appropriate measurement of total legume N. These factors are addressed below.

3.3.3. TIMING OF PLANT SAMPLING

Since leaf fall during reproductive development can contain 40–80 kg N/ha [4, 8], LegDM and NLeg are ideally determined at around the time of peak biomass (during mid-podfill for many crop legumes) before there is substantial loss of senescent leaves from the crop canopy (as indicated by the closed symbol in Fig. 1). Even if fallen leaves are collected during grain filling, often small losses of total plant N occur between physiological maturity and grain harvest (Fig. 1). It is important to realize that if only the standing biomass at grain harvest is used (as indicated by the open symbol in Fig. 1), measures of NLeg and Nfix will be underestimated, determinations of NHI are likely to be overestimated, and the calculation of the contribution of legume BNF to the N balance will be underestimated.

TABLE III. NET NITROGEN BALANCES FOR EXPERIMENTAL TREATMENTS IMPOSED ON CROP LEGUMES

(calculated on the basis of the amounts of shoot nitrogen fixed (Nfix) and the amounts of nitrogen removed in the seed (NLs) at grain harvest[a])

Experimental variable and crop species		NLeg (kg N/ha)	Ndfa[b] (%)	Nfix[c] (kg N/ha)	NLs (kg N/ha)	NHI[d] (%)	N balance[e] (kg N/ha)
Variety	*Groundnut*						
	-Virginia bunch	319	65	206	198	62	+8
	-Early bunch	326	62	206	237	72	–34
	Field pea						
	-L82	136	66	90	105	77	–15
	-Dundale	126	79	99	82	65	+17
Growth season	*Soybean*						
	-Early wet	328	72	236	178	54	+58
	-Late wet	107	72	77	83	78	–6
Year	-1980	259	70	181	180	69	+1
	-1982	323	72	233	272	84	–39
	-1984	309	79	244	246	80	–2
Farming system	*Faba bean*						
	-Rainfed	119	74	88	61	51	+27
	-Irrigated	144	75	108	64	44	+44

[a] Data collated from field trials undertaken in Australia, groundnut and field pea variety studies [10]; faba bean [1]; Thailand, soybean [10]; and Denmark, field pea [6].

[b] The proportion of legume N derived from atmospheric N_2.

[c] Nfix = (NLeg×Ndfa)/100.

[d] The harvest index for N = (NLs/NLeg)×100.

[e] N balance = (Nfix – NLs).

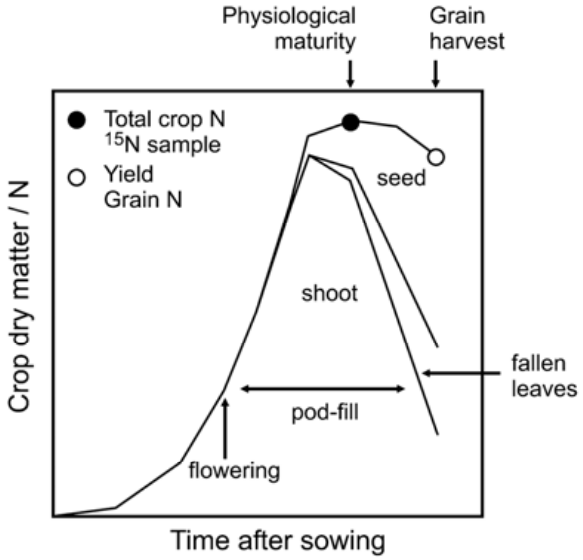

FIG. 1. Typical pattern of dry matter and nitrogen accumulation by a crop legume. The ideal timing for sampling to measure peak crop biomass and tissue-nitrogen content for determinations of crop nitrogen and plant nitrogen-15 composition to estimate the proportion of the legume nitrogen derived from the atmosphere (Ndfa) is indicated by the solid symbol (●). The measurement of seed yield at grain harvest to allow calculation of the harvest index of nitrogen is shown by the open symbol (○). (Adapted from Ref. [3].)

TABLE IV. MEASUREMENTS OF CROP BIOMASS AND ESTIMATES OF ERROR USING DIFFERENT SAMPLING TECHNIQUES[a]

Method of sampling	Mean crop biomass (t dry matter/ha)	Coefficient of variation (%)
Four randomly selected plants	9.5	39
0.3 m row (0.225 m^2)	9.6	42
1 m row (0.75 m^2)	7.0	19
27 m row (20 m^2)	6.7	9

[a] Data derived from a soybean crop grown with 0.75 m row spacing [11].

3.3.4. QUANTIFYING LEGUME BIOMASS

Measurement of the legume biomass (LegDM) is one of the greatest potential sources of error in calculating NLeg and Nfix in the field.

3.3.4.1. Experimental trials

Hunt et al. [11] evaluated the effect of the sampling protocol on the precision of estimates of shoot dry matter for soybean. Their findings (Table IV) indicated that even though the coefficient of variation was increased slightly using a 1 m row sampling regime (0.75 m^2) compared to a large subplot harvest of 27 m (equivalent to 20 m^2), similar mean determinations of crop biomass were obtained by both sampling techniques. However, reduction of sampling size to a 0.3 m row, or use of four individual plants (combined with a measure of plant population to calculate dry matter per m^2), increased variation by more than

fourfold. The 0.3 m and four-plant samples also significantly overestimated shoot dry matter (Table IV). This bias persisted with different samplers or technicians, even though the procedure used incorporated random selection of samples. It was assumed that the investigators subconsciously and consistently selected larger plants.

Therefore, the use of small sample areas or individual plants should be avoided. Experimental replicates must be designed to be large enough to accommodate a reasonable sample size, but still avoid errors due to 'edge' effects in the outside 0.5 m around the perimeter of individual plots. There is a need also for at least two samplings: once to measure peak biomass and another at maturity to determine seed yield (Fig. 1). If the resulting land requirements for four or more replicates appear too large for the area available, it would be preferable to sacrifice experimental treatments or comparisons rather than cut back on sampling size and hence lose precision.

3.3.4.2. Measurements in farmers' fields

Although reliable data may be obtained from experimental trials if they are designed and sampled with care, it can be exceedingly challenging to obtain a representative estimate of the amount of dry matter accumulated by commercial legume crops growing in fields that range in size from less than 1 ha to more than 300 ha. The strategy employed by Schwenke et al. [12] when surveying the symbiotic performance of farmers' dryland (rainfed) legume crops (using the ^{15}N natural abundance technique) and calculating the N balance of north-eastern Australian cropping systems entailed harvesting three 1 m rows (collecting non-legume weed materials as non-N_2-fixing reference plants within the same area) at each of ten different sampling points located at regular intervals along a fixed series of transects running diagonally across each field in a "W" shape (care was taken to avoid obvious 'headland' areas). The actual area sampled was calculated from in-field measurements of row spacing, but in all cases the area sampled in each replicate was at least 1 m^2. Experience from previous surveys showed this system to be preferable over alternative random or square-grid sampling protocols. One potential problem with such non-random plant sampling is that in heterogeneous crops (e.g. on fields that are drought affected) there may be no plants at the predetermined sampling point, but the data will reflect reality without the interference of operator bias.

3.3.4.3. Coping with large samples

If the legume material collected is too large and bulky to handle, either in terms of logistics in transport or subsequent drying, reasonable biomass determinations are still possible using the following protocols:

— Cut the shoots from at least 0.5–1 m^2, weigh and record the total fresh weight with a hanging balance in the field. The balance may be supported on a vehicle or tripod, and should be capable of weighing up to 10–20 kg of material in 50 g or 100 g gradations.
— From the bulk material, subsample sufficient individual plants to obtain an accurate measure of fresh weight (e.g., 5–10 plants to weigh 200–500 g fresh weight) with another hanging balance or battery operated balance capable of weighing up to 2–5 kg in 10 g or 20 g gradations.
— Measure and record the subsample fresh weight immediately while in the field; place the plants in a bag that clearly identifies the plot number, treatment, date of sampling and other key information.

— Upon return from the field, place the subsamples in an oven at 70–80°C for at least 48 h, and then reweigh.
— Appropriate conversion factors can then be derived from these data by determining the ratio of the dry weight to fresh weight for each individual subsample. When applied to the total fresh weight determinations of the original large field samples, these conversion factors will allow total plant dry matter to be estimated.
— The dried subsamples can later be ground and analysed for N and ^{15}N (if using a ^{15}N based methodology to estimate Ndfa).

Although it is important to base crop biomass and grain yield determinations on as large a sampling area as possible, reliable determinations of crop N and Ndfa can still result from analyses of the remaining small subsamples, since tissue-N content and ^{15}N composition tend to be far less variable than measurements of crop dry matter (e.g. Table V).

3.3.5. MEASUREMENTS OF TOTAL LEGUME NITROGEN

An underlying source of error in most calculations using Eqs (1)–(6) has been the basis of how NLeg and Nfix have been determined. The main sources of fixed N returned to the soil as a result of legume cropping are via fallen leaves and vegetative residues following grain harvest, and from the nodulated roots (Table I), and possibly also root exudates [13–15]. Yet most published data on the accumulation of legume N and N_2 fixation have relied almost solely on measures of above ground biomass N, and the contributions of below ground sources of N have either been ignored or grossly underestimated when N balances have been constructed in the past.

TABLE V. EXAMPLES OF DATA VARIABILITY WHEN DETERMINING CROP BIOMASS, TISSUE-N CONTENT AND THE PROPORTION OF LEGUME NITROGEN DERIVED FROM THE ATMOSPHERE (Ndfa) FOR FARMERS' LEGUME CROPS USING DIFFERENT SAMPLING PROTOCOLS[a]

Crop	Sampling area (m^2)	Coefficient of variation (%)		
		Shoot biomass	N content	Ndfa
Field pea	0.25	22	7	6
	0.5	10	7	5
	1.0	9	6	5
Faba bean	0.25	52	12	12
	0.5	38	11	12
	1.0	29	10	13

[a] Mean values derived from ten replicates of 1 m^2 were 7.5 and 7.8 t dry matter/ha for shoot biomass, 2.7 and 2.3% for N concentration, and 84 and 66% for Ndfa (determined using ^{15}N natural abundance) for commercial field pea and faba bean crops, respectively, growing under dryland conditions in south-eastern Australia (M.B. Peoples, unpublished data).

Various studies using a range of ^{15}N based techniques and non-isotopic approaches now suggest that N either associated with, or derived from, the turnover and decomposition of nodulated roots of crop legumes can represent 30–50% of the total plant N (e.g. Refs [16-19]).

Consequently, the more traditional shoot-based measurements, such as those presented in Table II, underestimate the total inputs of fixed N, and the net impact of legume cropping on the N balance of agricultural systems is likely to be much larger than suggested in Table III [1, 17]. This has major implications for our understanding of the potential contributions of legumes to the N economies of agricultural systems and can adversely affect management of those systems.

3.3.5.1. Estimating below ground nitrogen using non-isotopic methods

The procedure most commonly used to calculate below ground N (BGN) is based on the amounts of N detected in roots recovered from soil. Even when plants are grown in pots in the glasshouse, it is extremely difficult to collect all of the nodules and roots from a growing substrate. It is certainly unrealistic to expect anything more than partial recovery of roots from soil for field-grown legumes. Even assuming that it is possible to collect complete, intact root systems, this approach will still not include a measure of N deposited in the legume's rhizosphere, or the turnover of fineroot and nodule N that occurs during growth [7, 13, 14]. Thus, this method will always underestimate total BGN.

An alternative strategy can be applied to estimating BGN in glasshouse studies where amounts of a uniform and homogenous soil can be accurately weighed into pots. Soil N per pot at the beginning of the experiment can be subtracted from total soil N measured at harvest, and BGN calculated from the net N gain during legume growth. This procedure can be further refined if Ndfa is also determined and the amount of soil N in shoots can also be accounted for [18]. Theoretically, a similar approach could be applied in the field. However, since below ground inputs of fixed N are likely to represent less than 100–200 kg N/ha in a background of several tonnes of soil organic N, and in view of the large number of soil cores that would be required to quantify the small incremental change in soil N with any precision given the inherently heterogeneous nature of soil chemical and physical characteristics, the construction of N budgets to determine the contributions of BGN is rarely feasible for short term field studies [9].

3.3.5.2. Estimating below ground N using nitrogen-15 based methodologies

Because of the difficulties and errors associated with estimating BGN using non-isotopic techniques, various ^{15}N based methodologies have been developed in recent years. In these approaches, either the plant or the soil is ^{15}N labelled and the distribution of ^{15}N or changes in ^{15}N enrichment in the roots and root zone soil are quantified after a period of time.

3.3.5.2.1. Using nitrogen-15 to determine contributions of fixed nitrogen

Two basic procedures have been utilized:

— $^{15}N_2$ has been provided to nodulated roots in enclosures or cuvettes to specifically label fixed N (e.g., Ref. [20]), and
— inoculated plants are grown in ^{15}N enriched soil, and the below ground contributions of fixed N is determined from the dilution of soil ^{15}N [18, 21].

3.3.5.2.2. Root feeding of nitrogen-15

This consists of exposing part of the root system to a ^{15}N solution and recovering the label in the shoot, other non-exposed parts of the root, and soil (e.g. using a split-root system [13, 14], or feeding adventitious roots [22].

3.3.5.2.3. Shoot labelling with nitrogen-15

A key requirement of shoot labelling is that the whole plant (i.e. shoots and roots) is enriched with ^{15}N and that any ^{15}N detected in the soil is specifically associated with the roots.

The ^{15}N has been applied to shoots:

— as a foliar spray of urea [23],
— by immersing attached leaflets or petioles in vials containing enriched ammonium sulphate [14] or urea [15–18, 22],
— via a cotton wick inserted into a hole in the stem and linked at the other end to a reservoir of labelled urea [15,24], or
— by injecting enriched urea into the plant stem [17, 22].

All three isotopic approaches summarized above (i.e. using ^{15}N to identify fixed N, root feeding and shoot labelling) have been developed for particular purposes and have their own distinct advantages and limitations. It is unlikely, therefore, that any one technique will necessarily be broadly applicable to all legume species. Some of the techniques require complex and expensive equipment (e.g. gas-tight enclosures), considerable pre-experimental preparation (e.g. to obtain uniformly ^{15}N enriched soil) or specific plant morphological requirements (e.g. woody or hollow stems), while others result in disturbance of the plant–soil system (split-root technique), provide only short term measures ($^{15}N_2$ feeding), pose potential risks of soil contamination of applied ^{15}N (spray application) or are subject to different interpretations depending upon what control is used (e.g. comparison of the ^{15}N dilution of enriched soil by a legume treatment with bare soil or a non-legume).

3.3.5.2.3.1. Protocols for nitrogen-15 shoot feeding

The most straightforward and technically least demanding procedure appears to be ^{15}N labelling of leaflets or petioles [15]. This procedure has been applied in glasshouse studies and field trials involving both cool-season [14, 17–19, 25] and warm-season crop legumes [17, 19], pasture species [15] and green manures [22]. When shoot labelling has been compared to either alternative ^{15}N based procedures (growing inoculated legumes in ^{15}N enriched soil, or root feeding), or non-isotopic techniques (total mass balance, or derived from the physical recovery of root fragments) under controlled conditions, estimates of BGN obtained with shoot feeding were generally similar to those from other methodologies except for those calculated by physical recovery, which give very much lower values [18, 22].

Major criteria for the shoot labelling of legumes to determine BGN are that:

— the fed ^{15}N is translocated throughout the plant to enrich both above and below ground parts;
— the method of ^{15}N feeding is relatively rapid and convenient, and does not have deleterious effects on the plant;
— the ^{15}N in the root zone soil is derived only from the roots and nodules;

— the ^{15}N enrichment of root derived N in the soil is relatively uniform and is related to the enrichment of recovered roots; and
— the ^{15}N levels in recovered plant parts and in the soil are sufficiently high for isotopic analysis.

3.3.5.2.3.2. Form of nitrogen-15

Urea is the most commonly selected source of fed ^{15}N because it is a non-polar, undissociated molecule of low salt index and carries a high concentration of N relative to its mass [15]. It is also readily absorbed by plant tissues to be hydrolysed to ammonium carbonate by plant urease. The labelled ammonium is rapidly synthesized into amino acids, which are subsequently transported to the rest of the plant. However, urea can also be toxic to plant tissues at high concentrations. Therefore, when using ^{15}N-urea shoot-feeding procedures, the challenge is to supply sufficient label while avoiding tissue necrosis, which may interfere with uptake and/or subsequent translocation of the ^{15}N out of the fed organ. The result could be low and variable enrichments of ^{15}N in the target tissues. Several studies suggest that a urea concentration of around 0.5% (wt/vol.) is a reasonable compromise between securing a sufficiently high level of ^{15}N enrichment whilst minimizing the extent of tissue damage for a range of legume species [15, 19]. To ensure a detectable enrichment of roots and soil, highly enriched urea (98–99 at.% ^{15}N excess) is generally used.

3.3.5.2.3.3. Labelling procedures

Leaf feeding of ^{15}N: The leaflets of species such as faba bean, mung bean, pigeon pea and soybean are large enough for leaf-flap feeding, described by Pate et al. [27]. The selected attached leaflet is first placed in a Petri dish containing water so that it is fully submerged. A narrow "V" — with the point centred on the mid-vein close to the leaf tip — is then cut out to form a flap. The flap is immediately inserted into a small plastic tube containing 0.2–1.0 mL of 0.5% ^{15}N urea, and kept in place using a flexible non-porous plug of material (e.g. Teristat® putty or BluTack®) around the petiole. This material serves to seal the top of the tube to prevent evaporative losses or spillage, and to attach the tube to a small wooden stake placed next to the leaf. Alternatively, the tip of a leaflet (or all leaflets of a compound leaf for pasture species such as clovers) can be cut and placed in a vial of ^{15}N urea and sealed as described above. All of the ^{15}N solution is generally taken up within a few hours. If the ^{15}N urea remains in the vial overnight, the procedure can be repeated using another leaflet.

Petiole feeding of ^{15}N: Petioles of species such as chickpea, mung bean, pigeon pea and soybean are sufficiently long for the ^{15}N solution to be supplied through them (once the leaflets are detached), although the procedure may differ slightly for each species. With mung bean, only the middle trifoliolate need be removed from its petiole. With pigeon pea, petiole connections to all three trifoliolates need to be cut. For chickpea, leaflets near the tip of the petiole are removed, leaving some towards the bottom of the petiole. In all cases, the tip of the petiole is cut under water before being placed in a small tube containing ^{15}N urea, with subsequent procedures as described above.

Feeding protocols and sampling strategies: The limited data available suggest that leaf-feeding methods enrich the roots more than does petiole feeding [15, 19]. Feeding of ^{15}N using either leaf-flap or petiole techniques is best carried out with young plants, which appear to take up solution more rapidly and to apportion relatively more label to their root systems than do mature plants. With faba bean, there appeared to be no effect of fed-leaf position on the ^{15}N enrichment of the roots, whereas with chickpea, roots were more highly enriched

when the leaves at the base of the stem were fed; with both species, multiple feedings of ^{15}N tended to increase root enrichment [19].

Since the shoots of fed plants may be more highly enriched than the below ground parts, it is essential that contamination of the soil by senesced and fallen shoot material be prevented; otherwise the key assumption that all the ^{15}N detected in the root zone soil is derived only from the roots and nodules will be invalid. To minimize the risk of contamination, highly enriched fed leaves or petioles should be removed a few days or weeks after feeding. In glasshouse studies, the pots can be monitored daily and any fallen shoot material collected and removed. In theory, the same procedure could also be used for field trials, but at remote field sites that might be visited only periodically it may be preferable to place nets or mesh under the canopy of labelled crops to capture senesced leaves and fallen petioles to prevent their contact with, or their incorporation into, soil, so that they can be easily collected [15].

If plants are grown in PVC cylinders or pots, measurement of total ^{15}N recovery requires the shoots to be removed and retained for further analysis, and the contents of the pot simply upended onto a sheet of plastic for sampling. In field experiments, groups of plants are usually grown within enclosed microplots (see Section 3.3.6) and all the soil is removed to a certain depth manually and/or soil-cored. In either case, roots need to be recovered from the growing medium, the total soil volume weighed and (because of the large volume) mixed for subsampling. The shoots, recovered roots and soil are subsequently dried at 70–80^{o}C and analysed for N concentration and ^{15}N enrichment.

3.3.5.2.3.4. Calculations

Often it is more convenient to report small enrichments in ^{15}N in soil as $\delta^{15}N$ or parts per thousand (‰) relative to the ^{15}N composition of atmospheric N_2 (i.e. 0.3663 at.% ^{15}N):

$$\delta^{15}N = 1000 \times \frac{\text{atom\% } ^{15}N_{sample} - 0.3663}{0.3663} \quad (7)$$

Calculations of BGN from analysis of ^{15}N in recovered roots and soil assumes that all ^{15}N excess in the soil originated from the ^{15}N enriched root material. The ^{15}N data can be used to provide estimates of BGN and interpreted in several ways using different assumptions. The most commonly used approach assumes that the ^{15}N enrichment of all root-derived N in soil is the same as the enrichment of recovered roots (e.g. [16]). Thus, the amount of root-derived N in soil (BGN_{soil}) can be calculated from the specific relationship between ^{15}N excess and total N (i.e. mg ^{15}N excess per g root N) of recovered roots ($^{15}N_{RR}$/g) and the measured ^{15}N excess of the soil ($^{15}N_{soil}$):

$$BGN_{soil} = \frac{^{15}N_{soil}}{^{15}N_{RR}/g} \quad (8)$$

Total below ground N (BGN_{total}) is then calculated as the sum of the N measured in recovered roots (N_{RR}), and the estimated root-derived N in soil from Eq. (8).

$$BGN_{total} = N_{RR} + BGN_{soil} \quad (9)$$

However, active N_2 fixation generally results in localized dilution of the added ^{15}N in nodules, which can result in different enrichments between nodulated roots, unnodulated roots and nodules [15]. The importance of such differences can vary with legume species. For example, the data of Khan et al. [19] indicate that the relative enrichment values (enrichments of different below ground components relative to the nodulated tap root) for faba bean can be:

— nodulated tap roots (100),
— nodulated lateral roots (100),
— unnodulated roots (112), and
— nodules (62).

So, although faba bean nodules had a lower enrichment, values did not vary greatly between roots, regardless of whether they were nodulated or not. On the other hand, for a species like chickpea, which has a high proportion of BGN in nodules [7], the differences in enrichment can be much greater. Average values for chickpea in the study by Khan et al. [19] were:

— nodulated tap roots (100),
— nodulated lateral roots (111),
— unnodulated roots (156), and
— nodules (63).

It is clear that large differences in enrichment between below ground parts could introduce problems in data interpretation if the specific ^{15}N enrichment (i.e. mg ^{15}N per g root N) detected in the recovered root material is taken to be representative of all of the unrecovered root N remaining in the soil, particularly when dealing with situations where there might be a large nodule mass and/or the nodules are not uniformly distributed on the roots down the soil profile. In other studies, stratified root sampling appears to have countered this problem (e.g., Ref. [16]). Such an approach is not always possible in heavy clay soils because of the difficulty in recovering roots and nodules at depth (e.g., Ref. [16]). Therefore, BGN may be estimated using a second approach, which is to assume predominantly crown nodulation of the plants. Thus, recovered roots would be nodulated, while unrecovered roots and root-derived material remaining in soil would be without nodules. If the ratios of unnodulated root to nodulated root enrichments have been previously determined (e.g. 1.12 for faba bean and 1.56 for chickpea [18]), then these values could be used to adjust calculated BGN (termed adjusted ^{15}N shoot-labelling). Thus, Eq. (8) might be modified so that the BGN_{soil} for faba bean and chickpea, for example, would be derived by multiplying the ^{15}N enrichment of the recovered root samples ($^{15}N_{RR}$) by 1.12 and 1.56, respectively.

A third approach to estimating BGN is to assume that the distribution of ^{15}N throughout the plant reflects the partitioning of total plant N. In other words, the proportion of the total plant N associated with, or derived from, the nodulated roots would be calculated by comparing the amounts of ^{15}N present in the soil ($mg^{15}N_{soil}$) and recovered root ($mg^{15}N_{RR}$) with the total amount of ^{15}N detected both above ground ($mg^{15}N_{Shoot}$) and below ground:

$$\%BGN = 100 \times \frac{\text{mg } ^{15}N_{soil} + \text{mg } ^{15}N_{RR}}{\text{mg } ^{15}N_{soil} + \text{mg } ^{15}N_{RR} + \text{mg } ^{15}N_{shoot}} \quad (10)$$

Since this calculation is concerned only with the ^{15}N that has been translocated around the plant, the $mg^{15}N_{shoot}$ value used in Eq. (10) should exclude any ^{15}N excess still remaining in the fed leaves after their excision.

3.3.6. CASE STUDY

3.3.6.1 Site, treatments and sowing details

This case study is from a field experiment undertaken by Khan et al. [25] on the New South Wales Agriculture Liverpool Plains Field Station, Breeza (31°11'S, 150°25'E), in eastern Australia. Wheat was grown over the site in 1995 and 1996. The soil (0–20 cm depth) was an

alkaline (pH7.56 in $CaCl_2$) grey Vertosol of heavy clay texture (57% clay, 24% silt and 19% sand). Soil organic C and total N levels to 10 cm depth were 1.02% and 0.11%, respectively, and there was 102 kg nitrate-N/ha in the top 1.2 m of soil at sowing.

The design of the main experiment was randomized complete blocks replicated four times. The individual treatment plot size was 35 × 10 m. Faba bean (cv. Fiord) and barley (cv. Kaputah) were sown in 32 cm (barley) or 64 cm rows (faba bean) on 5 June 1997. Seeding rates were 65 kg/ha (barley) and 100 kg/ha (faba bean). Faba bean was inoculated just prior to sowing with the appropriate inoculant: Group E incorporating *Rhizobium leguminosarum* bv. viciae SU303. Starter fertilizer (9.4% N, 20.5% P, 2.2% S and 2.5% Zn) was incorporated at sowing at a rate of 60 kg/ha.

3.3.6.2. Plant labelling with nitrogen-15

Following seedling emergence, a metal microplot frame measuring 0.5 × 0.64 m was pushed into the ground to a depth of about 30 cm in each faba bean replicate to prevent below ground lateral losses of applied ^{15}N. Each microplot contained eight faba bean plants. The microplot plants were each fed 0.2 mL of 0.5% ^{15}N urea (98 at.% ^{15}N) five times during vegetative growth commencing 62 days after sowing, with final feeding on day 96. The total amount of ^{15}N applied to each microplot was 18.0 mg. Procedures for ^{15}N shoot-labelling using a leaf-flap were as described above. The fed leaves were removed within 2–3 weeks of feeding.

3.3.6.3. Harvest details

Fallen leaves were collected regularly to ensure that ^{15}N in the above ground parts did not contaminate the soil in the microplots. Replicate plots were harvested at maximum plant biomass, just prior to physiological maturity on day 155. Shoots, including any fallen leaves and petioles collected during the study, recovered root fragments and all soil to 25 cm depth were retained for dry matter, %N and ^{15}N determinations.

Eight soil cores (8 cm diameter) at 25–45 cm depth were also sampled from each microplot, for dry matter, %N and ^{15}N determinations. Four of the cores were taken from within the plant rows and four from between them. Values for the cores were extrapolated to the microplot area (0.32 m^2) to account for N and ^{15}N in the total soil volume at 25–45 cm depth.

Cores (4 cm diameter) were also collected from outside the microplots (i.e. in the unenriched main plots) to determine the %N and natural ^{15}N abundance of soil at the site. Shoot dry matter, %N and natural ^{15}N abundance values were measured for both faba bean and barley from three 0.5 × 0.64 m areas (total of 0.96 m^2) taken from each main plot. At plant maturity (20 November), further areas were cut at ground level for determinations of grain dry matter yield and N content.

3.3.6.4. Plant and soil analysis

The dried plant (70°C for 48 h) and soil (40°C for 48 h) material was coarsely ground in a Wiley mill (1 mm sieve), subsampled and then milled to a powder with a ring grinder (Rocklabs Pty Ltd, Auckland, New Zealand). The total N content and ^{15}N enrichment values 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, Crewe, United Kingdom).

3.3.6.5. Calculations

The reliance of faba bean upon atmospheric N_2 for growth (Ndfa) was determined by comparing the $\delta^{15}N$ values of the faba bean and barley material collected from the unenriched areas of the main plots as [3]:

$$\%\text{Ndfa} = 100 \times \frac{x - y}{x - B} \quad (11)$$

where

x is the $\delta^{15}N$ of shoots of the non-N_2-fixing barley reference plants deriving all of their N from the soil,
y is the $\delta^{15}N$ of the faba bean shoots, and
B is the $\delta^{15}N$ of shoots of faba bean plants deriving all of their N from fixation (–0.39‰) [12].

Estimates of BGN were calculated from the ^{15}N enrichment data in three ways:

— assuming that the ^{15}N characteristics of the root N still in soil were identical to that of the recovered root according to Eqs (8) and (9),
— applying the "adjusted ^{15}N shoot-labelling" approach to account for an uneven distribution of nodules as proposed in Section 5.2.3.4.,
— based on the amounts of ^{15}N measured above and below ground using Eq. (10).

3.3.6.6. Results

3.3.6.6.1. Nitrogen-15 enrichments and calculation of below ground nitrogen

The ^{15}N contents, expressed as $\delta^{15}N$ units, of faba bean and soil to 45 cm depth for both the microplots and unenriched areas of the trial are presented in Fig. 2. Enrichments of intact roots and shoots were 674 and 537‰, respectively. Microplot soil in 0–25 cm depth had an enrichment of 18‰, compared with 6.1‰ in the soil outside the microplots (natural abundance level). Enrichment in the 25–45 cm section of the microplot soil of 8.7‰ was just above the natural abundance level (6.3‰). Total recovery of the applied ^{15}N was calculated to be 91% based on the amounts of ^{15}N detected in the various plant and soil fractions (Table VI).

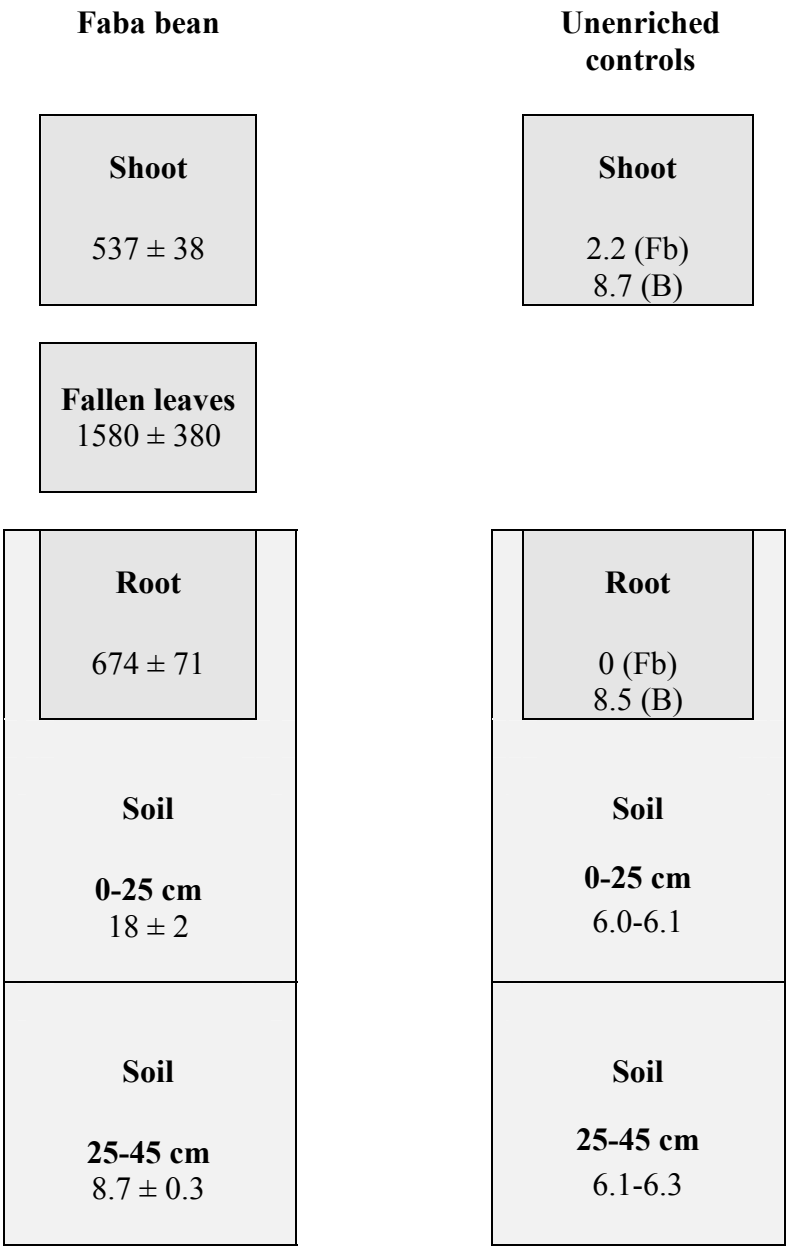

FIG. 2. The $\delta^{15}N$ ± s.e (‰) of shoots, fallen leaves, recovered roots and soil (0–25 cm and 25–45 cm) for nitrogen-15 shoot-labelled faba bean grown in (0.32 m^2) microplots in the field at Breeza, NSW. The $\delta^{15}N$ ± s.e (‰) value of the unenriched shoots and roots and soil (0–25 cm and 25–45 cm) for faba bean (Fb) growing outside the microplots and for barley (B) are also shown

TABLE VI. NITROGEN-15 IN PLANT PARTS AND SOIL, AND RECOVERY OF APPLIED NITROGEN-15 FOR SHOOT-LABELLED FABA BEAN

^{15}N in							Recovered (%)
Shoots	Roots	Bulk soil[a]	Core soil[b]	Fallen leaves	Fed leaves	Total	
(mg)							
10.2	0.32	3.67	0.43	0.74	0.97	16.4[c]	91

[a] 0–25 cm.
[b] 25–45 cm.
[c] The amount of ^{15}N transported out of the fed leaves = (16.4 – 0.97) = 15.4 mg.

An estimate of the amount of root N remaining in the microplot soil (1.70 g) was calculated on the basis of the specific ^{15}N enrichment of the recovered root material using Eq. (8) (Table VII). The final calculation indicated that BGN represented 25% of the total faba-bean plant N (Table VIII). If the estimate of BGN was recalculated assuming uneven nodulation, the adjusted root N equivalent in soil was 1.54 g and the BGN value was 24%. Based on the sum of the amounts of ^{15}N present in the recovered root (0.32 mg) and soil (3.67 + 0.43 mg) and the total amount of ^{15}N translocated from the fed leaves (15.4 mg, Table VI), the third estimate of BGN calculated from Eq. (10) was 29% of the total plant N. While all three ^{15}N based estimates of BGN were similar (24–29%), these values were considerably higher than one calculated by comparing the amount of root N that could be physically recovered from the heavy clay soil at the experimental site with the N measured in shoots and fallen leaves (BGN = 2%, Table VIII).

TABLE VII. CALCULATING ROOT-NITROGEN EQUIVALENTS IN THE SOIL FOR NITROGEN-15 SHOOT-LABELLED FABA BEAN

Recovered root[a]			Soil (0–45cm)[a]		
N (g)	^{15}N (mg)	mg ^{15}N/ g N	N (g)	^{15}N (mg)	Root N equivalent (g)[b]
0.13±0.01	0.32±0.03	2.47±0.26	134.6±2.29	4.10±0.66	1.70±0.25

[a] Values (± s.e.) are expressed per microplot.
[b] Calculated using Eq. (8).

TABLE VIII. CALCULATING BELOW GROUND NITROGEN (BGN) AS A PERCENTAGE OF TOTAL PLANT DERIVED NITROGEN FOR NITROGEN-15 SHOOT-LABELLED FABA BEAN GROWN IN 0.32 m^2 MICROPLOTS

Shoot + fallen leaves N	Recovered root N	Root N in soil	Total plant N	BGN (% of total N)
(g)				
5.52 ± 0.70[a]	0.13 ± 0.01	1.70 ± 0.25	7.35 ± 0.79	25

[a] Values (± s.e.) are expressed per microplot.

TABLE IX. SHOOT DRY MATTER (DM), NITROGEN CONTENT AND NITROGEN-15 NATURAL ABUNDANCE, AND GRAIN YIELD FOR FIELD GROWN FABA BEAN AND BARLEY

Species	Shoot[a]				Grain[a]	
	DM (t/ha)	N (kg/ha)	$\delta^{15}N$ (‰)	Ndfa (%)[b]	Yield (t/ha)	N (kg/ha)
Faba bean	5.66±0.83	168±24.7	2.18±0.44	72 ± 5.0	3.35±0.55	123±20.0
Barley	8.72±0.99	88±10.6	8.69±0.34		5.54±0.61	74±8.7

[a] Values for shoot and grain derived from four replicates of 0.96m^2 collected from the unenriched areas of the main plots.
[b] Calculated using Eq. (11).

3.3.6.6.2. Estimates of inputs of fixed nitrogen and calculations of nitrogen balance

The mean level of ^{15}N natural abundance measured for barley was considerably higher than that for faba bean (Table IX, Fig. 2), resulting in estimates of Ndfa for faba bean of 72%. Both faba bean and barley produced moderately high amounts of shoot dry matter (Table IX), given that the growing season was characterized by below average rainfall (224 mm for June–November (average: 295 mm)). Just 49 mm were recorded for the four months of June through September, compared with average rainfall for the period of 170 mm. Grain yields of barley (5.5 t/ha) and faba bean (3.4 t/ha) were slightly above average for the region.

Values for shoot and grain N and %Ndfa were combined with the average of the three shoot-labeling estimates of BGN (26%) to construct N budgets (Table X). Using just above ground (AG) data, the N balance for the crop was –2 kg N/ha. This is not surprising, given the close similarity between the calculated NHI (i.e. 100 × 123/168 = 73%) and Ndfa (72%). If it is assumed that the shoot derived estimate of Ndfa also reflected that of the whole plant (AG + BG), then inclusion of an estimate for the amount of fixed N below ground in the calculations resulted in a N balance of +40 kg N/ha.

TABLE X. CALCULATION OF NITROGEN BUDGETS FOR FABA BEAN BASED ON ABOVE GROUND (AG) VALUES ONLY AND ON BOTH ABOVE AND BELOW GROUND (BG) VALUES

Parameter	AG only (kg N/ha)	AG + BG (kg N/ha)
Shoot N	168	168
BGN	*	59
Estimate of total crop N	168	227
(Ndfa)	(72%)	(72%)
N fixed (Nfix)	121	163
Grain N (NLs)	123	123
N balance (Nfix – NLs)	–2	+40

3.3.6.7. Discussion

The objective of this case study was to quantify BGN of rainfed faba bean and to use the values to determine N balances for the cropping system. The BGN value for faba bean in this study of 26% was less than the values of 37% for glasshouse-cultured plants from a parallel study and 41% for irrigated field-grown plants, both derived using ^{15}N shoot-labelling [17, 18]. Turpin et al. [27] reported a BGN value of 21% for rainfed field-grown plants using physical recovery and wet sieving. It should be remembered that such non-isotopic methods do not include N that is in the soil as a result of rhizodeposition or the turnover of fine root and nodular N that occurs during growth. Thus, the BGN value of Turpin et al. [27] is likely to be an underestimate.

Whilst we are confident in the estimate of BGN of 26% for faba bean under the conditions prevailing in this field experiment, it is also apparent from the range of published estimates that BGN can differ in response to the conditions of plant culture. The allocation of assimilates to above and below ground parts is influenced by edaphic and environmental conditions. Species also differ in their relative responses to water stress. Therefore, the root:shoot ratio can change from species to species and even among cultivars of the same species. In our field experiment, conditions were dry for much of the season, with only 49 mm rainfall recorded between June and the end of September. Given these conditions, it might have been reasonable to expect root:shoot ratios to differ from those of plants grown under ideal conditions in a glasshouse or with irrigation.

Having said that, it would be useful to have ’default‘ BGN values that could be applied to studies in which there is no accompanying experimentation to quantify BGN. We propose using a value of 34% derived as averages of this ^{15}N study and those previous investigations referred to above, as the ’default‘ value for faba bean. Clearly, the use of such values would be preferable to underestimating or ignoring the BGN component and assuming that roots and nodules contribute little or no N to the N economies of faba bean to rotational systems where it is included.

This case study involving faba bean confirmed that a high proportion of N is associated with, or derived from, roots and nodules and that this fraction represents a potentially important pool of N that is often grossly underestimated or ignored in calculating N balances. Future

descriptions of the contribution of N_2-fixing legumes to the N economies of agricultural systems should benefit by including more accurate ^{15}N derived estimates of BGN.

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CHAPTER 4

USE OF TRACER TECHNOLOGY FOR THE MANAGEMENT OF ORGANIC SOURCES

R. HOOD-NOWOTNY

FAO/IAEA Agriculture and Biotechnology Laboratory,
Agency's Laboratories Seibersdorf,
International Atomic Energy Agency,
Vienna

C. VAN KESSEL

University of California,
Davis,
California,
United States of America

B. VANLAUWE

Tropical Soil Biology and Fertility Institute of CIAT,
Nairobi,
Kenya

This chapter covers the management of organic sources as evaluated by nuclear technologies. Firstly (4.1) the production in the field and glasshouse of plant material labelled with nitrogen-15 — for investigation of organic sources of crop nutrition — is described in detail, including that from trees. Also discussed are approaches for labelling animal manure with nitrogen-15, and for dual labelling of plant material with isotopes of carbon and nitrogen. In the second session (4.2) a brief review of soil nitrogen (N) dynamics and its relationship to carbon (C) is provided, including kinetic aspects of nitrogen mineralization from organic matter. The utility of nitrogen-15 tracers as a tool in related studies is furthermore described. Finally (4.3) a historical perspective of organic-matter management in agriculture is provided, and factors affecting organic-residue decomposition in soil are discussed. The relationship between organic-resource quality and nitrogen release for plant uptake, as well as potential contributions of organic inputs to soil fertility and crop yields, are also discussed, with particular emphasis on tropical agriculture.

4.1. ISOTOPE LABELLING METHODS FOR EVALUATING CONTRIBUTIONS OF ORGANIC SOURCES TO PLANT NUTRITION

R. HOOD-NOWOTNY
FAO/IAEA Agriculture and Biotechnology Laboratory,
Agency's Laboratories Seibersdorf,
International Atomic Energy Agency,
Vienna

4.1.1. LABELLING OF ORGANIC RESIDUES WITH NITROGEN-15

Production in the field and glasshouse of plant material labelled with ^{15}N — for investigation of organic sources of crop nutrition — is described in detail, including that from trees. Also discussed are approaches for labelling animal manure with ^{15}N, and for dual labelling of plant material with isotopes of carbon and nitrogen. The indirect technique for measuring nitrogen derived from organic sources is discussed with special reference to pool substitution; where ^{15}N label and residues are applied simultaneously, the isotope dilution and A-value techniques are inappropriate for determination of plant N uptake from organic sources.

Crop residue and green manure studies using the direct method are relatively simple. Labelled green manure can be easily obtained by fertilizing with ^{15}N and the above or below ground material then added to unlabelled soil. The percentage N in the next crop derived from added residue (Ndfr) is calculated using the equations below [1].

$$\%\text{Ndfr} = \frac{\text{atom }\%^{15}\text{N excess in the crop}}{\text{atom }\%^{15}\text{N excess in the residue added}} \times 100 \quad (1)$$

The quantity of N derived from the residue can be calculated as follows:

$$\text{Ndfr (kg)} = \frac{\%\text{Ndfr}}{100} \times \text{N in the crop} \quad (2)$$

Percentage N derived from the applied residue can be calculated as follows:

$$\frac{\text{Ndfr (kg)}}{\text{amount of N added as residue (kg)}} \times 100 = \%\ \text{N recovery} \quad (3)$$

4.1.2. PRODUCTION OF LABELLED MATERIAL

Application of ^{15}N fertilizer to soil or sand (pot grown plants) is the simplest way of labelling plant material; however, some precautions should be taken. The plant material should be evenly labelled, therefore the ^{15}N tracer should be present throughout the growth period. Thus, split or multiple applications of ^{15}N fertilizer are recommended. Target enrichment of the residue should be considered when designing such production systems. For most studies, an enrichment of approximately 0.5–1 at.%^{15}N excess is adequate. However, higher enrichments may be required if emission spectrometry is to be used to determine the isotope ratios of the crop. Measurement considerations are outlined in Table I. If the material will be subsequently used as animal feed in manure experiments or release of N studied over a number of cropping seasons, then again the target enrichment of the plant material needs to be higher and the necessary target enrichment needs to be estimated. When labelling an N_2-

fixing plant, it is necessary to take account of the dilution effect caused by fixation of unlabelled N and to be aware that levels of N higher than 30 kg/N ha may inhibit N_2 fixation [2].

The labelling strategy should take account of how best to label the plant material and establish the desired final enrichment of the material. Some questions that may need to be asked are:

— Will the labelled material be added to a crop to determine N derived from residue?
— Will the material be fed to animals to obtain labelled manure and then applied to the crop to determine N derived from manure?
— Is the plant being labelled to estimate N benefit to the following crop?

Although production of labelled residues in the field is ideal, it can be expensive. Much of the fertilizer may be immobilized by the soil microbial biomass, lost by leaching, etc. Labelled materials can also be produced by growing plants in sand or other inert media, supplemented with the nutrient solution supplemented with ^{15}N, either in the greenhouse or in pots outdoors. Effort should be made to produce material that has characteristics similar to those of field-grown plants.

The use of ^{15}N labelled residues in agronomic studies has enhanced our understanding of N turnover in soil. Examples of residue studies are shown in Table II.

It is possible to use previously generated data and observations to make initial estimates of amounts of ^{15}N required for labelling. This will facilitate planning and experimental design. An example is presented in which leaf residues from soybean were added to a maize crop to determine the legume N contribution to the cereal.

4.1.2.1. Example 1

The objective was to determine the amount of N in a maize crop derived from 1 tonne/ha of dry soybean residue. From previous studies it was known that soybean residue usually has approximately 2% N and a leafy dry biomass yield of 3 tonnes/ha. It was assumed that the soybean derived 65% of its N from the atmosphere and 10% from the soil.

The experimental design had five maize microplots of 2 × 2 m (1 m^2 sampling area each) for the isotope part of the experiment. Nitrogen derived from residue was estimated to be around 10% using data collected from the literature. All ^{15}N measurements would be made using the emission spectrometer.

TABLE I. MEASUREMENT CONSIDERATIONS FOR ^{15}N LABELLING

	Measurement by optical emission spectrometry	Measurement by isotope ratio mass spectrometry
Ideal range of measurement	0.2–2.0 at.%^{15}N excess	0.1–0.5 at.%^{15}N excess
Error	1%	0.4% natural abundance 0.5‰
Range of measurement	0.2–80 at.%^{15}N excess	0.003–10 at.%^{15}N excess

TABLE II. STUDIES IN WHICH THE NITROGEN-15 DIRECT TECHNIQUE HAS BEEN USED TO MEASURE NITROGEN DERIVED FROM RESIDUE

Following crop	Residue added	Residue N	Ndfr[a] (%)	Ndfr	Ref.
Sorghum (*Sorghum bicolor*)	*Acacia saligna*	94 kg/ha	28	6 ± 2 kg/ha	[3]
Maize (*Zea mays*)	*Casuarina equisetifolia*	500 mg/pot	21	100 mg/pot	[4]
Maize	Groundnut (*Arachis hypogaea* 'Tainan 9')	100 kg/ha	8.3	9.6 kg/ha	[5]
Maize	Groundnut (*A. hypogaea* 'LKK60-1')	110 kg/ha	9.3	8.7 kg/ha	[5]
Winter barley (*Hordeum vulgare*, first season)	Pea (*Pisum sativum*)	82.8 kg/ha	15	10.9 kg/ha	[6]
Oilseed rape (*Brassica napus oleifera*)	Pea	82.8 kg/ha	13	11.4 kg/ha	[6]
Rhodes grass (*Chloris gayana*)	Siratro (*Macroptilium atropurpureum*)	391 mg/pot	51	46.1 mg/pot	[7]
Rhodes grass	Soybean (*Glycine max*)	222 mg/pot	20	11.7 mg/pot	[7]

[a] N derived from residue.

4.1.2.1.1. Calculations

Initially, enrichment in the maize must be more than 0.2 at.%^{15}N excess for accurate measurement with the emission spectrometer; our target enrichment was 0.5 at.%^{15}N excess in the maize crop. We then calculated the amount of label residue required.

Five plots of 2 × 2 m (20 m^2) were to receive labelled residue at a rate of 1 tonne/ha, thus the required amount was:

Microplot area/10 000 (area of a hectare in m^2) × residue addition rate in tonnes,

i.e. $\frac{20}{10{,}000} \times 1 = 0.002$ tonnes or 2 kg of residue per 20 m^2

To allow for unforeseen circumstances such as inadequate yields, it is advisable to plant more than is required. We aimed for 5 kg per 20 m^2.

We then calculated the area to be planted to produce enough residue. From previous experiments, we knew that soybean yields leafy biomass of approximately 3 tonnes/ha, i.e. 3000 kg per 10 000 m^2 or 0.3 kg per m^2. Thus, to obtain 5 kg we needed to plant $\frac{5}{0.3}$, i.e. 16.7 m^2; as we allowed for additional material, we chose a convenient 4 × 4 m plot size.

As we were to grow the material in the field and plants at the edges of the plots take up N from unlabelled soil, to ensure that all material had the same enrichment it was necessary to leave a boundary area of 0.5 m around the plot. Therefore, plots of 5 × 5 m (25 m^2) were set up to receive ^{15}N.

Next we determined the target enrichment of the soybean residue. The literature indicated Ndfr values for maize of approximately 10%. Again, our target enrichment was approximately 0.5 at.%^{15}N excess in the maize. As the maize was to receive no additional fertilizer and does not fix N_2, it had only two sources of N:

Ndfr + Ndfs (N derived from soil) = N maize

%Ndfr + %Ndfs = 100

The ^{15}N abundance of the maize is a function of the abundances of the two sources of N, the soil and the residue. The final abundance of the crop depends on the proportions or percentages of N derived from residue and soil, and their respective abundances. This can be expressed mathematically as:

%Ndfr(*a res*) + %Ndfs(*a soil*) = 100(*a crop*)

where *a* is ^{15}N abundance.

If we use enrichment or at.% excess values and assume, for simplicity, that soil has a natural abundance of 0 at.% excess (i.e. background), the soil term disappears and the equation becomes:

%Ndfr(*e res*) = 100(*e crop*)

In our example, the target enrichment (*e*) of the crop is 0.5 at.%^{15}N excess; we assume that %Ndfr will be around 10. Therefore, by rearranging the equation we can calculate the target enrichment for the residue crop:

$$\frac{100(e\ crop)}{\%\text{Ndfr}} = (eres)$$

$$\frac{100(0.5)}{10} = \frac{50}{10} = 5\%$$

Thus, the target enrichment of the soybean residue was 5 at.%^{15}N excess.

We then needed to calculate the enrichment of ^{15}N fertilizer to apply to the soybean to obtain the target enrichment in the residue. In soybean it is assumed that approximately 65% of the N is from fixation (Ndfa) and approximately 10% of the N is from the soil; therefore, 75% is from unlabelled sources and 25% is from the labelled fertilizer. Soybean has three sources of N; where Ndff is N derived from fertilizer:

%Ndff + %Ndfs + %Ndfa = 100

%Ndff(*a fert*) + % Ndfs(*a soil*) + Ndfa(*a air*) = 100(*a crop*)

However, there is only one labelled source and the other sources are at natural abundance or 0.0 at.% excess, i.e.:

%Ndff(*e fert*) = 100(*e crop*)

We can then calculate the required (*e fert*) or enrichment of the fertilizer by rearranging the above equation, knowing that the target enrichment of the soybean crop is 5 at.%^{15}N excess and % Ndff is 25%:

$$\%\text{Ndff}(e\ fert) = 100(e\ crop)$$

$$\frac{100(e\ crop)}{\text{Ndff}} = (e\ fert)$$

$$\frac{100(5)}{25} = 20$$

Thus the fertilizer should be enriched at 20 at.%^{15}N excess.

As N applications are known to inhibit N_2 fixation and we wanted the residue to have normal characteristics of soybean, we added the labelled fertilizer at rates of less than 30 kg N/ha. To obtain uniformly labelled plants, the ^{15}N is applied over the growing season; in this example a three-way split application (post emergence, one month later and prior to pod filling) of 20 kg N/ha per application was chosen.

The N required per 25 m^2 was calculated as follows:

$$\frac{\text{Plot area (m}^2)}{10{,}000} \times \text{N rate (kg/ha)} = \frac{25}{10{,}000} \times 20 = 0.05 \text{ kg N/plot}$$

The amount of ammonium sulphate $(NH_4)_2SO_4$ required to provide 0.05 kg N is calculated as follows:

The atomic weights of the components of $(NH_4)_2SO_4$ are N = 14, H = 1, S = 32 and O = 16; therefore, the molecular weight is 14 + 14 + 8 + 32 + 64 = 130 and the weight of $(NH_4)_2SO_4$ required per plot is

$$\frac{130}{28} \times 0.05 = 0.232 \text{ kg}$$

It is not necessary to account for the difference in molecular weight due to the ^{15}N; however, if this were a study of fertilizer use efficiency, then it would be necessary.

Thus, in total, 3 × 0.232 kg (232 g) or 0.696 kg (696 g) of 20 at.%^{15}N excess $(NH_4)_2SO_4$ was required to label the plots. Extra solution should be prepared to allow for spillage and other accidents, e.g. in this example it is recommended that at least 800 g of 20 at.%^{15}N excess be ordered.

To label the plot evenly, it is best to subdivide it with strings into eight 1.25 × 1.25 m subplots and apply the fertilizer in solution. Dissolve the fertilizer in an adequate amount of water so that it can be accurately measured out in the field using a measuring cylinder. In this example it is recommended that the fertilizer be made up in 2 L of water, so that one would measure out 230 mL in the field using a 250 mL measuring cylinder (with the 230 line on the measuring cylinder marked with a waterproof marker). This allows for spillages (to be avoided if possible) but also ensures that the last plot receives the full amount of label. Thus:

$$230 \times 8 = 1840 \text{ mL}$$

Therefore, 232g of $(NH_4)_2SO_4$ dissolved in 1840 mL of water is the minimum requirement. It would be better to make up 2 L of solution in a volumetric flask, using the following amount of $(NH_4)_2SO_4$:

$$\frac{2{,}000}{1{,}840} \times 232 = 252 \text{ g (at 20 atom \%}^{15}\text{N excess.}$$

In the field, measure out the required volume of solution using a measuring cylinder, add it to a watering can with a sprinkling rose attached, and make it up to 3–5 L depending on plot size. Apply the solution across the plot with a sweeping motion attempting to cover the whole plot evenly. Repeat until all microplots are labelled.

Repeat this process for each labelling event. Record all relevant data such as planting and sampling dates, times of application, weather, etc.

4.1.2.1.2. Harvesting

The area for harvest is marked out, discarding material in the boundary area; the material in the subplots was collected and fresh weight recorded. The methods of sample preparation, drying and field application are determined by the objectives. If necessary, the plants are separated into appropriate parts, e.g. leafy biomass and pods. Plant material is usually roughly chopped, mixed well and dried at 50–60°C for 3–4 days in a large drying oven; it is stored in airtight containers in sealed plastic bags until required. Subsamples are analysed for N concentration and ^{15}N abundance. Samples should also be archived for any further analysis that may be needed.

When applying the residue to the maize crop, uniformity of application is important, but it is also important that it reflect the practice being emulated. Again, this is dependent on the objectives. If Ndfr is to be determined under regular agricultural practice, and if normal practice is to chop and leave the residues on the surface, then the experimental residues should be processed and applied likewise. If wind is likely to move the residues, it may be necessary to fasten a plastic net or mesh over them until they are suitably degraded.

4.1.2.1.3. In summary

— Determine the experimental objective;
— Calculate the quantity of label material required;
— Calculate the target enrichment of material required;
— Calculate the amount and enrichment of ^{15}N fertilizer required;
— Develop a strategy that ensures uniform labelling;
— Ensure that the harvesting strategy guarantees uniform labelling;
— Dry and store material appropriately.

4.1.3. NITROGEN-15 LABELLING BY GROWING MATERIAL IN SAND CULTURE

The high price of ^{15}N often limits experimental work; for example, manure studies require large quantities of labelled forage. Application of fertilizer N to soil invariably leads to immobilization by microbial action and to losses, making much of the precious ^{15}N tracer unavailable for plant uptake. An alternative way of labelling material is to grow it in an inert medium such as sand or vermiculite with nutrient solution added. Usually it is best to use a low-N solution, especially for N_2-fixing plants, to produce materials similar to those from field-grown plants. Labelled nutrient solutions can be applied daily, thus ensuring evenly labelled material. If the plants are grown outside and not under glasshouse conditions, they are usually more similar to field crops. Again, all relevant data pertaining to production of the labelled material should be recorded.

Table III shows stock nutrient solutions, based on the Long Ashton formula [8], that have been used in a number of experiments. To make up the stocks, it is necessary to use a precise

and accurate balance and volumetric flasks. To make the solutions up to final required dilution, pour 5 L of water into a container and add 20 mL of each solution (measured using a measuring cylinder and rinsed with distilled water between solutions) into the container mix and make up to 10 L. This avoids problems of precipitation. The stocks in Table III are enough to make 250 L of solution. As the cost of the ^{15}N label is high, you may want to make up only 100 mL of KNO_3 stock solution, i.e. dissolve 20.5 g KNO_3 in 100 mL, but again using 20 mL of stock for the final solution, providing a total of 50 L of solution. This solution is relatively low in N; however, further reduction in N concentration may be achieved by further substituting KCl for KNO_3.

The nutrient solution and stock solutions should be stored in the dark to prevent algal growth. Another tip is to cover the pot surface with plastic beads (diameter ~3 mm), available from plastics manufacturers. Although not essential, the beads curtail evaporation from the pots and prevent algal growth.

4.1.3.1. Example 2

The objective was to determine N in wheat derived from glasshouse-grown alfalfa (lucerne) residue. It is known that alfalfa has the potential to fix large quantities of N_2; therefore, the Ndfa (N derived from atmosphere) was assumed to be 80% with an N concentration of 2.5%. Alfalfa was to be applied to four microplots of wheat of 3 × 3 m (9 m^2), at the rate of 2 tonnes/ha (0.2 kg/m^2). With a wheat yield of 5 tonnes/ha, we know from the literature that Ndfr is in the region of 20%. Previous experiments indicate that a plastic tray (30 × 20 × 5 cm) yields 750 g of dry biomass of alfalfa at 40 days, with each tray requiring 200 mL/day of nutrient solution. For analysis by mass spectrometry, the target enrichment of the wheat was 0.2 at.% ^{15}N excess.

TABLE III. STOCKS FOR SAND-CULTURE NUTRIENT SOLUTION

Reagent	Quantity of reagent (g)	Volume of water to dissolve the reagent (mL)
$CaCl_2.2H_2O$	20.4	100
$MgSO_4.7H_2O$	92.0	500
KNO_3	101.0	500
$NaH_2PO_4.2H_2O$	52.0	500
FeNaEDTA	9.2	1000
$MnSO_4.4H_2O$	1.115	1000
$CuSO_4.5H_2O$	0.125	1000
$ZnSO_4.7H_2O$	0.145	1000
H_3BO_3	1.55	1000
NaCl	2.95	1000
$NaMoO_4.2H_2O$	0.0605	1000

The amount of dry residue was calculated as microplots × area per microplot × application per unit area, i.e. 4 × 9 × 0.2 = 7.2 kg.

Each tray yielded 750 g of dry residue, therefore, at least ten trays $\left(\frac{7200}{750}\right)$ of alfalfa would be needed. To allow for losses, it would be wise to make up 12 trays. Each requires 200 mL of solution per day, i.e. 8000 mL for the 40 day period. Twelve trays would require 96 000 mL, i.e. 96 L of nutrient solution.

Five hundred mL of KNO_3 stock gives 250 L of nutrient solution; therefore, to obtain 100 L (rounded up from 96 L) of solution, we would require 200 mL of stock, i.e. 40.4 g of labelled KNO_3 dissolved in 200 mL of water.

The target enrichment for the wheat was 0.2% ^{15}N excess, and the Ndfr was estimated at approximately 20%. As the wheat will receive no additional source of labelled N, we know that the final abundance of the crop is a product of the %N derived from residues and its abundance and the percentage of N derived from soil and its abundance. Thus:

$$\%\text{Ndfr}(a\ res) + \%\text{Ndfs}(a\ soil) = 100(a\ crop)$$

As stated previously using at.% excess as the unit, assuming for simplicity that the soil is at natural abundance or 0.00 at.% excess, this equation becomes:

$$\%\text{Ndfr}(e\ res) = 100(e\ crop)$$

where e is at.% excess.

In our example, the target enrichment (e) of the wheat is 0.2 at.%^{15}N excess. Assuming that the Ndfr will be around 20%, by rearranging this equation the target enrichment for the residue crop may be calculated:

$$\frac{100(e\ crop)}{\%\text{Ndfr}} = (e\ res)$$

$$\frac{100(0.2)}{20} = 1\%$$

Thus the target enrichment of the alfalfa residue would be 1 at.%^{15}N excess.

To calculate the enrichment of ^{15}N fertilizer applied to the alfalfa to obtain the target enrichment, it is assumed that approximately 80% of the N will be from fixation. In the nutrient culture it is assumed that there is no source of N other than Ndfa (fixed N) and Ndff (labelled fertilizer N added); the pure sand does not provide N:

$$\%\text{Ndff} + \%\text{Ndfa} = 100$$

$$\%\text{Ndff}(a\ fert) + \%\text{Ndfa}(a\ air) = 100(a\ crop)$$

$$\%\text{Ndff}(a\ fert) = 100(a\ crop) - \text{Ndfa}\ (a\ air)$$

$$\%\text{Ndff} = 100 - 80 = 20\%$$

There is only one labelled source, therefore:

$$\%\text{Ndff}(e\ fert) = 100(e\ crop)$$

The required ($e\ fert$) or enrichment of the fertilizer may be calculated by rearranging this equation:

$$\frac{100(e\ crop)}{\text{Ndff}} = (e\ fert)$$

With a target enrichment of alfalfa of 1 at.%^{15}N excess and the %Ndff at 20%, the required enrichment of the fertilizer would be:

$$\frac{100(1)}{20} = 5 \text{ at.\%}$$

Thus, this experiment would require 40.4 g of 5 at.%^{15}N excess KNO_3 to label the alfalfa.

This example demonstrates the opportunity for substantially reducing the requirement for ^{15}N labelled fertilizer by using an inert substrate and a more sensitive instrument for measurement. Again, care must be taken to ensure uniform labelling and that the plants possess characteristics of field-grown crops.

4.1.4. LABELLING OF RESIDUES USING TREE INJECTION

This technique has evolved due to problems of labelling trees by having to label large quantities of soil. Injection is a relatively easy and effective method for the study of N transformations and cycling in undisturbed tree/soil systems, as shown in Figs 1 and 2 [4].

Labelled N is injected into the active xylem stream of the growing tree, followed by a period of equilibration; subsequent injections may be necessary, after which the labelled leaf biomass is collected. Large quantities of plant material are labelled relatively inexpensively; this also facilitates studies of below ground N input. Seiter and Horwath [5], who used this method to study alder (*Alnus sinuta*) in situ, showed that 18% of the ^{15}N injected was taken up by a maize companion crop, 12% of which came from the above ground fraction of the alder. By the end of two growth seasons, 80% of the injected ^{15}N was in the soil fraction. However, both above and below ground components only supplied 3% of the N recovered in the maize crop.

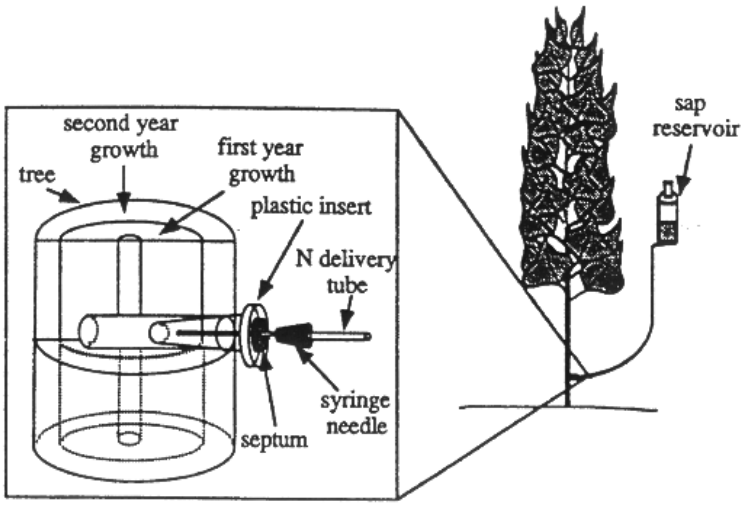

FIG. 1. Tree injection principle.

FIG. 2. Tree injection technique showing needle inserted into the trunk.

4.1.4.1. Practicalities

4.1.4.1.1. Use of nitrogen-15

This technique, pioneered by Horwarth et al. [4], allows assessment of the contributions of root material to N flux and organic matter, and allows large amounts of tree residue to be labelled at minimal cost.

4.1.4.1.1.1. Principle

A hole is drilled into the trunk and ^{15}N solution injected into the transpiration stream. The distribution of ^{15}N in the plant is determined by sampling throughout the canopy. This technique can be used to study above and below ground cycling on N.

4.1.4.1.1.2. Considerations

Species with ring-porous xylem are most suitable for this procedure, i.e. active xylem that is evenly distributed throughout the stem and not only on the surface. The location of the xylem can be determined by cutting a fresh stem and placing it in a shallow solution of basic fuchsin for several hours. Cut the stem and examine the cross-section; from this it is easy to determine where the water moves from the stain pattern. If xylem is present only on the surface of the trunk, the technique works less well.

4.1.4.1.1.3. Apparatus

— Electric drill
— Drill bit, 4 mm (type used for wood drilling)
— Two syringe needles
— 2 mL and 50 mL syringes
— Tube connectors
— Suba-Seal® septa, size 17

— Plastic pipe, 30 cm long, fitting onto the tube connector
— Paper punch
— pH meter or strips
— Autoclave or pressure cooker
— 1000 mL and 500 mL volumetric flasks
— Parafilm® sealant film
— Basic fuchsin indicator.

4.1.4.1.1.4. Reagents

— Nitrogen-15 solution, autoclaved at 120°C for 15 min (a 70 m*M* solution of $(NH_4)_2SO_4$:9.353 g of $(NH_4)_2SO_4$ dissolved in 1 L of distilled water). This gives a total of 0.204 g N per 100 mL of solution. Use between 10 mL and 100 mL of ^{15}N solution per tree, depending on size. Do not add more than 5% of the total tree N content, otherwise leaf 'burning' results.
— Artificial sap solution (5.0 m*M* KCl and 0.4 m*M* malic acid adjusted to pH5.4 with dilute potassium hydroxide (KOH) or sodium hydroxide (NaOH) solution, autoclaved at 120°C for 20 min).
— To prepare a stock sap solution, dissolve 3.75 g KCl and 0.54 g malic acid in 1 L of distilled water. To obtain the correct sap solution, dilute the stock solution 1:10 with distilled water and adjust to pH5.4 with dilute alkali (0.01 *M*).

4.1.4.1.1.5. Procedure

Cover the soil with aluminium foil and paper to protect it from spills. The technique relies on active transpiration, therefore it is best to make injections early in the morning when transpiration is maximum. Prior watering may increase the rate.

4.1.4.1.1.6. Pipe system preparation

Cut the body of a 2 mL syringe at approximate 2 cm and connect with a Suba-Seal® septum. Then connect about 20 cm of piping to the body of a 50 mL syringe, and on the other end attach a tube connector and a syringe needle. Attach the 50 mL injection system to the tree with tape and flush through with sap solution (not labelled with ^{15}N). Fill another syringe with unlabelled sap solution.

Measure the width of the trunk, label it at two thirds of the diameter with a small piece of tape and drill a hole at that location. Immediately after the hole is drilled, connect the syringe body/Suba-Seal® connector. Put a second needle in the Suba-Seal® septum, flush the system using the syringe filled with sap solution to expel all of the air, which should lead to solution coming from the second needle. Remove both needles.

Connect the needle with the 50 mL syringe system, mark the level of the sap solution and cover the open 50 mL syringe with Parafilm®. When uptake of sap solution is verified, add the ^{15}N solution to the syringe and keep topping up with the ^{15}N solution until the desired amount (10–100 mL) has been injected. Again mark the 50 mL syringe to determine the uptake volume. Top up the syringe with artificial sap solution to ensure that all of the ^{15}N enters the tree and continue until uptake stops. If there is substantial uptake, it may be best to take a plastic bottle and insert the end of the tube into this. Uptake of sap solution can be as much as 2 L. Repeated application of ^{15}N over the growth period should ensure uniform labelling.

4.1.5. LABELLING OF ANIMAL MANURE

Nitrogen-15 techniques have been used to determine N release from organic residues such as animal manure. Labelled plant material is fed to the animal, and the manure is collected and applied to the crop; the N derived from the manure (Ndfm) is determined using Eq. (4). The production of labelled manure is a complex and expensive operation. The manure must be evenly labelled both temporally and chemically. It is recommended that urine and faeces be collected separately. The advantage of this approach is that it allows direct measurements of uptake of manure N by plants and N loss by mass balance, facilitating investigation of management strategies.

$$\%\text{Ndfm} = \frac{\text{atom }\%^{15}\text{N excess in the crop}}{\text{atom }\%^{15}\text{N excess in the manure added}} \times 100 \quad (4)$$

This technique has been used to study plant N uptake from pig, goat, poultry and sheep manures [6–9]. Another approach is to label manure or urine by spiking with ^{15}N; urine is normally spiked with urea [10]. Stockdale and Rees [11] attempted this approach by labelling a variety of manures followed by anaerobic incubation; however, they concluded that distribution of the label in the manure was uneven, which led to difficulty in interpreting the results. Labelling of urine appears to be more successful. Bronson et al. [12] labelled sheep manure and showed that one third of the urine N was lost as ammonia gas on application to a sandy soil in summer in Australia.

In labelling manure, several factors should be taken into account. When an adequate quantity of feed has been accumulated, care is needed to collect the correct fraction of manure. Here we describe a number of details to obtain a homogenously labelled representative sample. Nitrogen is contained in several fractions [8]:

— Indigestible feed,
— Microbes from the rumen (approximately 17% [13]),
— Digestive secretions,
— Living and dead microbes from the intestine (approximately 33% [13]).

These fall into two pools, one slowly decomposable, consisting of undigested feed N and similarly enriched microbial N from the rumen, and a more rapidly decomposing pool consisting of living microbes, partly decomposed microbial tissues, digestive secretions and dead cells. Endogenous N dilutes the latter, which is less highly labelled than feed N or the slowly decomposable pool. Sørensen et al. [8] concluded that N release from the slowly decomposable pool was minimal in soil incubation experiments. He suggested that ^{15}N labelled manure can be a useful tool in studying the fate of manure N, but that recovery of organic ^{15}N gives only an approximate measure of actual recovery.

Sørensen et al. [8] obtained labelled manure by feeding a castrated sheep daily with 950 g DM of unlabelled hay for 7 days followed by ^{15}N labelled Italian ryegrass hay (4.5 at.% ^{15}N excess) for 9 days; faeces and urine were quantitatively collected separately, using plastic bags taped to the animal.

The urine and faeces from each 24 h collection were separately pooled and mixed, and stored at –18°C. The ^{15}N enrichments were determined after freeze-drying the material to prevent loss of urea N due to volatilization.

Based on experimental evidence, Sørenson and Jensen [9] suggested two strategies for labelling:

— for approximately 4 days, providing ^{15}N labelled feed and pooling faeces from days 2, 3 and 4, or
— feeding for a longer period and using only faeces sampled after 10–15 days.

Evaluation of the homogeneity of the manure is recommended [9] by incubation in quartz sand. This involves incubating 5 mg faecal N in 25 g of sand (then watered to 55% water-holding capacity) and measuring N mineralization in triplicate, by extraction of inorganic N using 2 *M* KCl (1:10, e.g. 25 g sand to 250 mL 2 *M* KCl) and measuring the ammonium and nitrate concentrations in the KCl extract.

He et al. [6] used highly ^{15}N labelled rice straw and cowpea stover as feed for young male goats and pigs, respectively, approximately 20 kg in weight at the start of the experiment. Goat faeces of 6.285 ^{15}N at.% and pig faeces of 6.823 ^{15}N at.% were obtained after 3 days of feeding.

4.1.6. DUAL LABELLING OF RESIDUES

Dual or multiple labelling of residues facilitates the study of mineralization of more than one element simultaneously. Dual labelling with ^{13}C and ^{15}N enables a more detailed understanding of soil mineralization processes [14–16]. It can be achieved either by growing plants in an ^{15}N labelled medium in an enclosed chamber continually supplied with or pulse-labelled with $^{13}CO_2$ (Fig. 3) [16]. Care must be taken to ensure that the material is uniformly labelled. Dual labelling with ^{14}C has also been reported [14, 17, 18], but there appear to be few publications using other types of multiple-labelling procedures for residue turnover studies.

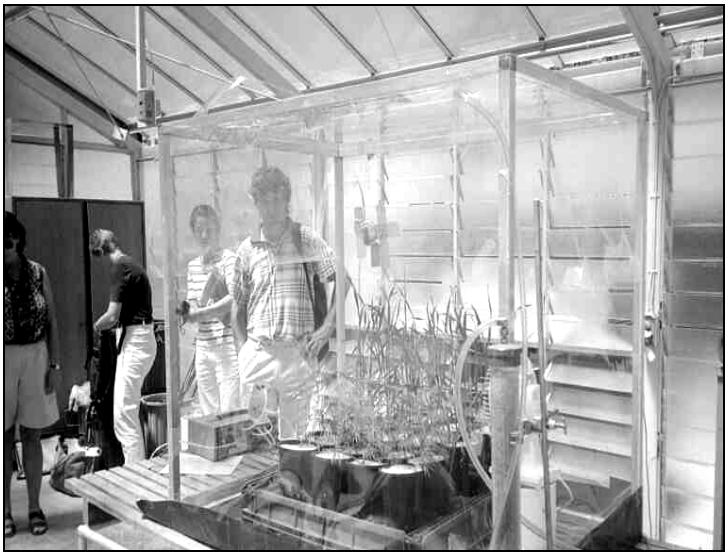

FIG. 3. Chamber for labelling plants with $^{13}CO_2$.

4.1.7. INDIRECT TECHNIQUE FOR MEASURING NITROGEN DERIVED FROM MANURE/RESIDUES

It is difficult to predict the availability of manure N to plants, since the processes of immobilization and mineralization, in addition to N losses, can occur simultaneously. Hatch et al. [19] reiterated this, stating that net mineralization values provide only limited information about the opposing processes of gross mineralization and immobilization that culminate in the release of N into the inorganic pool. The use of the stable isotope ^{15}N has significantly improved our understanding of N cycling in agricultural systems.

Indirect techniques can be used to study plant N uptake from organic residues or animal manures; however, precautions must be taken to avoid problems of pool substitution. The principle is that an inorganic ^{15}N tracer is added to the soil, and treatments with and without residues (no-residue controls) are set up. The no-residue control will have an ^{15}N abundance that reflects the soil ^{15}N pool, and the residue treatments generally have a lower ^{15}N due to the input of the unlabelled N from mineralization of the unlabelled residue. In practice, application of ^{15}N label and residues at the same time has been shown to cause errors associated with pool substitution [20]. Pool substitution is the process by which added labelled inorganic N (fertilizer) stands proxy for unlabelled inorganic soil N that would have otherwise been abstracted from a common pool that contains labelled and unlabelled N [21, 22]. However, the problem of pool substitution can be overcome if the soil is pre-labelled with ^{15}N and left to equilibrate for up to 6 months prior to the application of residues [23, 24].

Soil pre-labelling can be achieved by adding a carbon source and ^{15}N fertilizer simultaneously. This technique yielded good results in the greenhouse and compared well with the direct technique [23]. However, care must be taken to ensure that material of the correct C:N ratio is added as the pre-label and that the pre-labelling takes account of the inorganic N initially present in the soil. The ideal scenario is that the entire inorganic N is immobilized and fully incorporated into the soil microbial biomass. Only when the soil has returned to the initial levels of inorganic N concentration is the second phase of the experiment initiated, with addition of crop residue or manure. Experiments suggest that a C:N ratio of 1:24 is approximately correct for short term experiments [23]. Field studies have shown that, due to the difficulty of uniformly labelling the soil profile, this method of pre-labelling was not ideal. An alternative pre-labelling method was tested in which the ^{15}N fertilizer was applied to the crop preceding the one that received the residues. This allowed the fertilizer to be taken up by the initial crop and the ^{15}N to be distributed throughout the rooting zone. The above ground component was then removed and the soil left to equilibrate over winter. After this period, the second phase of the experiment was initiated, in which the manures were added. This method yielded comparable results to the direct technique. To achieve sufficient labelling in the following crop to allow calculation of Ndfr, applications of around 100 kg/ha of 10 at.%^{15}N are recommended to the previous crop. Microplots are recommended, but care should be taken to ensure minimal movement of soil.

The amount of N derived from manure (Ndfm) and %N recovery from the fertilizer — often referred to as fertilizer efficiency — can be calculated using Eqs (5–7) [1, 24, 25]:

$$\%\text{Ndfm} = \left(1 - \frac{\text{atom \% }^{15}\text{N excess}_{\text{ manure treatment}}}{\text{atom \% }^{15}\text{N excess}_{\text{ no-manure control}}}\right) \times 100 \quad (5)$$

$$\text{Ndfm} = \frac{\%\text{Ndfm}}{100} \times \text{N in crop} \quad (6)$$

$$\% \text{ N recovery} = \frac{\text{Ndfm}}{\text{amount of N applied as manure}} \times 100 \quad (7)$$

4.1.7.1. Pool substitution

If label and residues are added simultaneously, there may be a rapid decline in the ^{15}N abundance of the N pool and differential immobilization in treatments versus controls, leading to problems caused by pool substitution (defined in Section 4.1.7). The extent of the pool substitution effect or added-N interaction (ANI) is dependent on the immobilization capacity (i) of the soil, the initial inorganic N pool (P) and the quantity of labelled N fertilizer added (F), and is given as follows [26]:

$$\text{ANI} = \text{i}\left(\frac{\text{F}}{\text{P} + \text{F}}\right) \quad (8)$$

The immobilization of N on addition of organic material to soil is dependent mainly on the C:N ratio of material [27]. In the majority of soils, it is probable that any addition of N will lead to an initial immobilization of that N by the microbial biomass [21, 26]. The degree of immobilization is likely to be higher in soils where fertilizer and residues are added together than in those receiving fertilizer N alone [28], and thus cause problems with the A-value and isotope dilution methods. This may also explain why there is a lack of literature using these theoretically simple approaches.

The problems can be graphically demonstrated (Fig. 4), with the shaded area representing the ^{15}N label and the box area representing the size of the N pool. In the conventional ^{15}N dilution approach, two treatments are imposed: organic N added or no organic N added; simultaneously the soil is labelled with a ^{15}N fertilizer. When fertilizer is added alone, there is no immobilization (not strictly true but it will help explain the problem) and thus the whole amount of labelled and unlabelled N is available for plant uptake (Fig. 4(a)). Over a period of time, N from basal mineralization will be added to this pool.

When fertilizer and residues are added together, it is likely that there will be some immobilization by the microbial biomass, depending on the C:N ratio of the residue [21, 26]. Thus, if 50% of the inorganic N pool is immobilized, for example (Fig. 4(b)), only half of the N remains available for plant uptake. However, the N released from basal gross mineralization is likely to remain constant [29], thus resulting in greater dilution of the label. This dilution is due only to the pool substitution effect and not to additional N coming from an unlabelled source. This leads to erroneous estimates of Ndfr using the conventional isotope dilution approach [20].

In the proposed 'new' approach to the isotope dilution method, the soil is pre-labelled with ^{15}N until a stable abundance in the soil inorganic N pool is reached. Only then are treatments imposed, organic N added or no organic N added.

In theory, the N from mineralization will be at the same ^{15}N abundance as the inorganic N pool. When residues are added (Fig. 4(d)) or not added (Fig. 4(c)), the resultant N pool will have the same ^{15}N abundance in both cases, irrespective of the amount of immobilization that has taken place (assuming no N is coming from the residues). However, if N is coming from the residues, there will be a dilution of the resultant N pool. This is the dilution we are hoping to find, as this is a true dilution due to greater N in the pool and not due to pool substitution. Thus the conventional isotope dilution equations can be used.

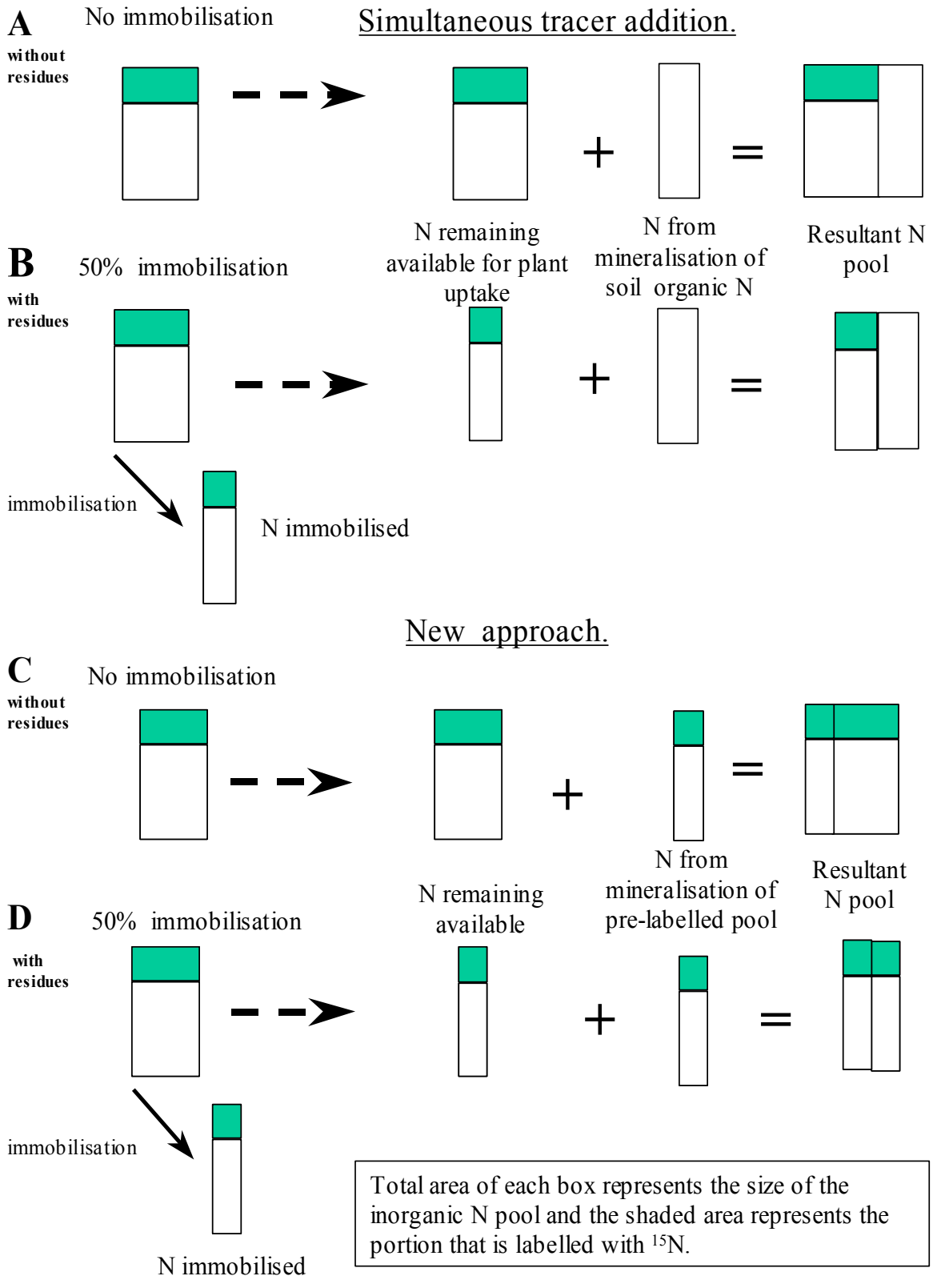

FIG. 4. Graphical representation of pool substitution.

Where plants have been growing for some time or seed N is a significant proportion of the final total N of the harvested plant, a correction factor must be included in view of the fact that, mathematically, seed N or initial plant N appears as a source of unlabelled N. This is done by calculating the ^{15}N abundance of the N increment [30].

This is not a completely new approach; treatments that stabilize the ^{15}N enrichment of plant available N have been used for measuring N_2 fixation using the isotope dilution technique [31]. One advantage of using ^{15}N dilution to measure plant N uptake from organic inputs is that it can be compared to the direct technique, to determine the validity of assumptions made.

4.1.7.2. Calculations

In the direct method, %Ndfr is calculated as in Eq. (1), whereas in the indirect technique %Ndfr is calculated as in Eq. (9).

$$\%\text{Ndfr} = \left(1 - \frac{^{15}\text{N atom \% excess}_{\text{treatment}}}{^{15}\text{N atom \% excess}_{\text{control}}}\right) \times 100 \quad (9)$$

In the indirect approach, where a correction for seed N or initial plant N is necessary, the correction is calculated as in Eq. (10). The correction is made both for the control and for the treatment as in Eq. (5).

$$\text{Corrected } ^{15}\text{N} = \left(\frac{\left(^{t=t}\text{N} \times ^{t=t}\text{N}^*\right) - \left(^{t=0}\text{N} \times ^{t=0}\text{N}^*\right)}{^{t=t}\text{N} - ^{t=0}\text{N}}\right) \quad (10)$$

$$\%\text{Ndfr} = \left(1 - \frac{\text{corrected } ^{15}\text{N atom \% excess}_{\text{treatment}}}{\text{corrected } ^{15}\text{N atom \% excess}_{\text{control}}}\right) \times 100 \quad (11)$$

where

$^{t=0}N$ and $^{t=0}N^*$ are total N and ^{15}N abundance of seed,
$^{t=t}N$ and $^{t=t}N^*$ are total N and ^{15}N abundance at harvest.

Nitrogen derived from residue expressed as an amount can be calculated as in Eq. (12) (same as Eq. (2)):

$$\text{Ndfr (mg)} = \frac{\%\text{Ndfr}}{100} \times \text{total N (mg)} \quad (12)$$

The fraction of N recovered from the residue can be calculated as in Eq. (13) (same as Eq. (3)):

$$\%\text{N recovery from residue} = \frac{\text{Ndfr (mg)}}{\text{N added as residue (mg)}} \times 100 \quad (13)$$

4.1.7.3. Experimental evidence of problems

In this case study from Hood et al. [20], the direct approach for estimating crop N uptake from ^{15}N labelled organic inputs was compared with two indirect approaches: ^{15}N dilution and A-value. In the first experiment, soils received 25, 50, 75 or 100 mg N/kg soil in the form of *Casuarina equisetifolia* residues in addition to $(NH_4)_2SO_4$ fertilizer, to give a total of 100 mg N/kg soil. A cross-labelling design was used: two matching sets of treatments were set up, identical in all respects but the position of the ^{15}N label. Maize plants were grown in the soils amended with residues for 11 weeks, and N derived from residue (Ndfr) was estimated using the A-value or the direct approache. The A-value method appeared to significantly overestimate %Ndfr compared to the direct method. In the second experiment, contrasting

residues were added to soil: faba bean (*Vicia faba* 'Minor'), alfalfa (*Medicago sativa)*, N_2-fixing soybean, non-fixing soybean, barley and maize. This also had a cross-labelling design; labelled and unlabelled residues were used. Maize plants were grown in these soils for 11 weeks, and %Ndfr was estimated using ^{15}N dilution and the direct approach. The ^{15}N dilution approach again overestimated %Ndfr compared to the direct method in this experiment (Fig. 5). Pool substitution appeared to be responsible for the discrepancies between the direct and indirect techniques. It was concluded that the ^{15}N dilution and A-value approaches, as used in these experiments (i.e where residues and ^{15}N label are added simultaneously), were not appropriate techniques for estimating N derived from organic residues in soils.

Values of %Ndfr obtained using the isotope dilution (simultaneous labelling) approach ranged from 22 to 35% and were significantly ($P > 0.05$) and consistently higher than the values obtained using the direct estimations of %Ndfr (Fig. 6).

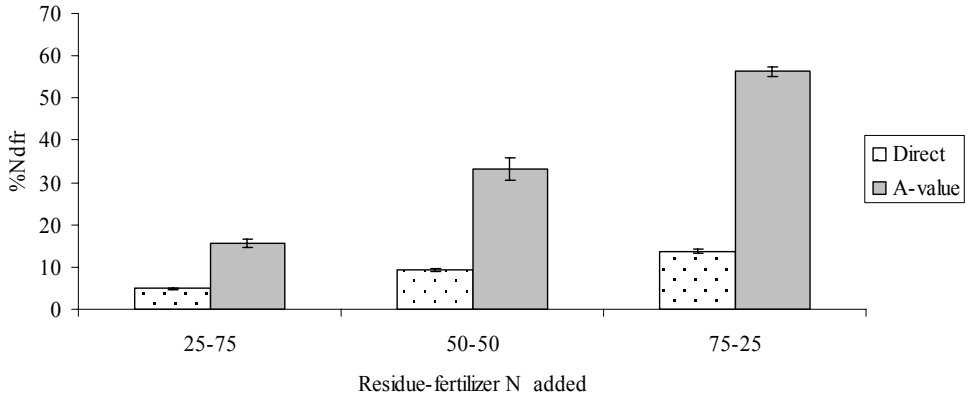

FIG. 5. Estimates of the amount of nitrogen derived from residues (data not shown) and %Ndfr obtained using the A-value approach, and estimates of the amount of nitrogen derived from residues and %Ndfr obtained using the direct approach.

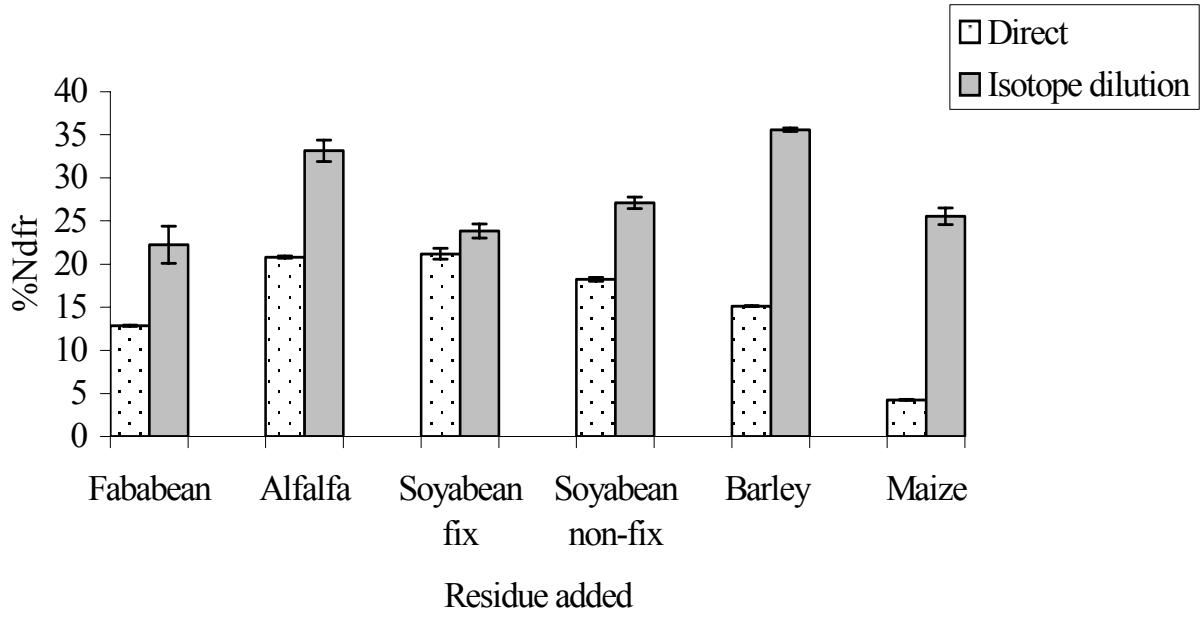

FIG. 6. Values of %Ndfr obtained using the direct and isotope dilution (simultaneous labelling) approaches.

It was hypothesized that, in these experiments, the immobilization capacity of soils with residues added differed from that of no-residue controls, i.e. less immobilization of inorganic N occurred in the no-residue control than in the with-residue treatments, resulting in a larger labelled N pool available for mixing with the unlabelled N from basal mineralization, leading to a more ^{15}N enriched pool than in the residue treatments. Conversely, in the residue treatments there was greater immobilization, making less labelled N available for mixing with a similar quantity of N from mineralization, thus resulting in lower ^{15}N abundance in the final inorganic N pool. The latter leads to higher estimates of Ndfr calculated using either the A-value or the isotope dilution approach compared to the direct method. Both the A-value and the isotope dilution methods assume that the ratio of available labelled fertilizer N to soil N remains constant in the controls and the treatments. In methods where residues and label are added to the soil simultaneously, this is not the case; therefore, the A-value and isotope dilution methods are invalid.

In summary, the isotope dilution and A-value techniques as used in these experiments (i.e. ^{15}N label and residues added simultaneously) are inappropriate techniques for determination of plant N uptake from organic residues, due to the effects of pool substitution.

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4.2. NITROGEN TRANSFORMATIONS AND TURNOVER IN SOILS AMENDED WITH ORGANIC SOURCES

C. VAN KESSEL
University of California,
Davis,
California,
United States of America

4.2.1. INTRODUCTION

A brief review of soil nitrogen (N) dynamics and its relationship to carbon (C) is provided, including kinetic aspects of nitrogen mineralization from organic matter. The utility of nitrogen-15 tracers as a tool in related studies is described, and factors that affect nitrogen mineralization are discussed, including the chemical characteristics of organic materials, soil and environmental factors and management practices. Routes of loss of nitrogen from soils amended with organic material are also described.

Of the nitrogen (N) on earth, 98% or 1.6×10^{23} g is present in the lithosphere. The N pools that comprise the most dynamic parts of the global N cycle link atmospheric N with N in terrestrial systems and the oceans. Whereas the oceans contain 2.3×10^{19} g of N and the atmosphere contains 3.8×10^{21} g, much smaller amounts exist in soil organic matter (9.5×10^{16} g) and in living biota (3.5×10^{15} g) [1]. In almost every terrestrial system, whether managed or unmanaged, soil organic matter serves as the largest potential source of N for plants. However, the vast majority of the N in soil organic matter, often close to 100%, is unavailable for plant uptake; non-N_2-fixing plants rely mainly on inorganic forms, i.e. NH_4^+ and NO_3^-, as their principal sources of N. Only through a cascade of various mineralization processes is soil organic N converted into inorganic N. Because the mineralization process is driven predominately by soil biota, factors like temperature and moisture availability strongly influence the rate at which organic N is converted into inorganic forms. The first inorganic N product is NH_4^+, which, under aerobic conditions, is converted by nitrifying bacteria to NO_3^-.

Soil microorganisms, which rely on organic substrates as their sources of carbon (C) for synthesis, are classified as heterotrophic organisms. In contrast, microorganisms that derive their C directly from CO_2 are classified as autotrophs. With regard to energy source, if soil microorganisms depend on a chemical source, e.g. soil organic matter, for energy, they are classified as chemotrophs. Bacteria that use light for energy are known as phototrophs. The vast majority of soil organisms relies on soil organic matter as their C and energy sources and belong to the category of chemo-heterotrophs. Therefore, mineralization of N from soil organic matter is closely associated with C cycling. An understanding of N dynamics during decomposition of soil organic matter or organic residues requires knowledge of C dynamics. For example, whether N in soil organic matter compounds remains immobilized or will be released in inorganic forms in the soil matrix depends on the N requirements of the microorganisms for growth, which in turn is controlled by C availability.

The amount of C in the soil organic matter pool is usually large. A particular soil that has a C content of 1% and a bulk density of 1.0 g/cm^3 contains 30 000 kg/ha of soil organic C in the top 30 cm of the profile. A soil that has a C content of 3% and a similar bulk density contains 90 000 kg/ha of organic C in the top 30 cm. With an average C:N ratio of 10:1, the amount of

organic N in the first example is equal to 3000 kg N/ha and in the second example it is 9000 kg N/ha. Because the C and N pools are so large, small changes in total soil C and N contents are difficult to detect. It is also clear that soil organic matter is a prime storage place for N. Some of that N is available within a short period of time, whereas most of it is present in a form that becomes available only at a slower rate and remains stored on a more permanent basis.

The use of ^{15}N tracers in plant and soil studies allows the determination of small changes in the size of a particular N pool, e.g. plant N uptake, microbial biomass N or release of inorganic N from plant residues. It may be possible that the total size of N in some of these pools, like microbial biomass N, remains constant. However, the incorporation of ^{15}N into the microbial biomass reflects turnover of the N pool. The use of ^{15}N tracers allows determination of the rate by which a particular N process occurs — e.g. mineralization, nitrification, immobilization or microbial and plant-N uptake — and construction of a total ^{15}N budget. Thus ^{15}N tracers can be of great assistance in further improving understanding of the mechanisms and dynamics of these processes.

4.2.2. KINETICS OF N MINERALIZATION

Nitrogen mineralization is defined as the transformation of N in the organic state into inorganic forms of NH_4^+ and NO_3^- (Fig. 1, Ref. [2]). Because N can be mineralized as well as immobilized, net and gross rates of N mineralization can be determined [3]. The net rate of N mineralization (ΔN) can be quantified using the non-^{15}N method known as the N balance approach and is equal to:

$$\Delta N = \Delta NH_4^+ + \Delta NO_3^- + \Delta plant + Nloss$$

where N loss can occur via leaching or gaseous emissions via NO, NO_2, N_2O, N_2 or NH_3.

Because it is difficult to measure all losses of N, the net rate of N mineralization is often determined by non-isotopic methods in an incubation experiment under laboratory conditions. As the N losses are assumed to be nil or small in the absence of plants, the net rate of mineralization becomes the net change in NH_4^+ and NO_3^- content during the incubation period. The amount of N mineralized is usually expressed in kg N/ha or mg/kg of soil.

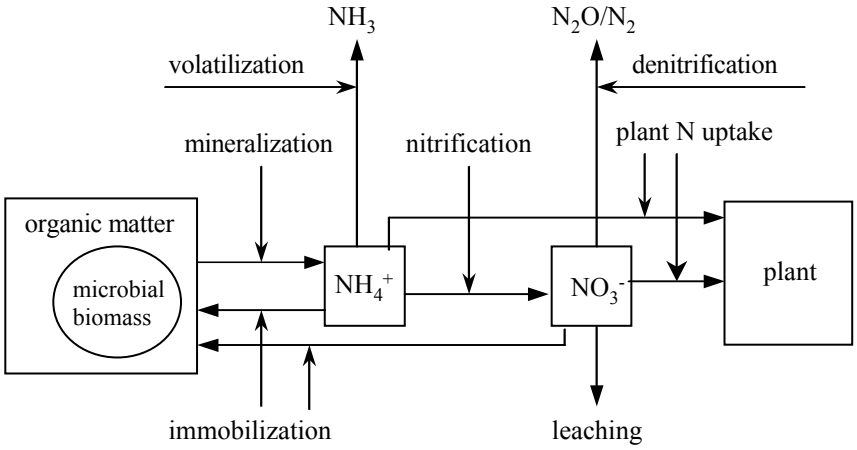

FIG. 1. Schematic overview of the major nitrogen cycling and transformation processes in a terrestrial ecosystem.

Of more significance is the rate of gross mineralization of N from soil organic matter or crop residues. Because the release or the immobilization of N is controlled mainly by the C:N ratio of the organic matter, soil organic matter with a narrow C:N ratio will lead to the release of inorganic N whereas organic matter with a wide C:N ratio will lead to an immobilization of inorganic N. Although it is not easy to determine the mineralization immobilization turnover of every pool of organic matter without carrying out a physical or chemical fractionation, the use of ^{15}N allows prediction of gross rates of mineralization immobilization and the ability to relate those rates to organic matter quality.

To determine gross mineralization of organic matter, changes in amount and ^{15}N enrichment of the NH_4^+ are followed over time (Fig. 2). A small amount of $^{15}N\text{-}NH_4^+$, added to the soil at time zero, is diluted by the $^{14}N\text{-}NH_4^+$ already present. If microbial biomass accumulates NH_4^+ or if the nitrifiers convert NH_4^+ into NO_3^-, the total amount of NH_4^+ in the soil will decrease, but the isotopic signature of the NH_4^+ will not change. The only way to change the isotopic signature of the NH_4^+ pool is through input of unlabelled $^{14}N\text{-}NH_4^+$ from organic matter via mineralization. When the rate of mineralization is high, the decline in the ^{15}N enrichment of NH_4^+ will be rapid. On the other hand, if the decline in the ^{15}N enrichment of NH_4^+ is slow, the rate of mineralization of organic matter is low. Some of the labelled and unlabelled NH_4^+ can be removed, but removal by itself does not lead to a change in the ^{15}N isotopic signature of the NH_4^+ pool.

Four conditions have to be met to make the analytical solution for gross mineralization valid [3]:

— None of the processes that increase or decrease the NH_4^+ pool (mineralization, nitrification, plant N uptake, microbial uptake) discriminate between ^{14}N and ^{15}N, and the subsequent use of $^{15}N\text{-}NH_4^+$ and $^{14}N\text{-}NH_4^+$ by the various processes is in proportion to the $^{15}N\text{-}NH_4^+$ and $^{14}N\text{-}NH_4^+$ present.
— The applied $^{15}N\text{-}NH_4^+$ is homogeneously mixed with the indigenous, unlabelled NH_4^+ pool.
— During the course of the experimental period, often limited to a few days or less, all rate processes follow zero order kinetics, i.e. the substrate is in such abundance that it does not limit mineralization.
— If $^{15}N\text{-}NH_4^+$ becomes immobilized, the ^{15}N is not remineralized. As the immobilization mineralization processes can be rapid, the period to determine gross mineralization is often short. For the first 7–14 days following the application of $^{15}N\text{-}NH_4^+$, remineralization of labelled N is considered to be negligible. Therefore, an incubation period of less than 1 week is advised and an incubation period as short as 1–3 days is commonly used to avoid violation of this and the previous condition.

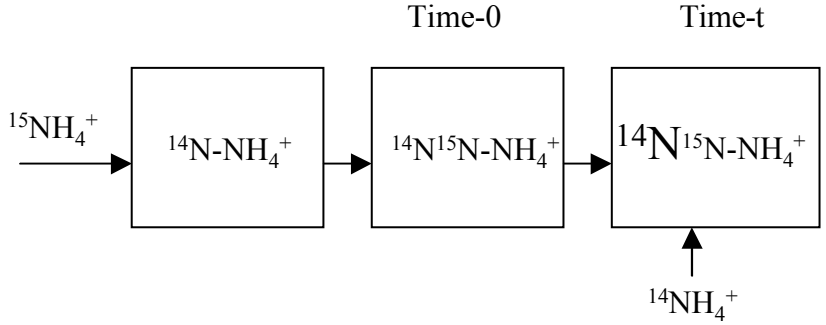

FIG. 2. Dilution of the $^{14}N^{15}N\text{-}NH_4^+$ pool with $^{14}NH_4^+$ from mineralization of organic matter.

4.2.2.1. Determination of gross nitrogen mineralization using nitrogen-15 ammonium

To determine the rate of gross mineralization, 100 g of soil is placed in a cup and $^{15}N\text{-}NH_4^+$ solution added. To disturb the soil as little as possible, it may be left in sampling cores. However, if the soil is not premixed, the requirement that the ^{15}N label be applied homogeneously throughout the soil sample (second condition above) will be more difficult to meet. If the soil is premixed and added to a cup, the $^{15}N\text{-}NH_4^+$ solution can be applied as a mist or solution. If cores are used, the $^{15}N\text{-}NH_4^+$ solution is injected with a syringe at multiple points throughout each core. The amount of $^{15}N\text{-}NH_4^+$ added is small (2 mg N/kg soil) and highly enriched (99 at.%^{15}N). The small amounts of NH_4^+ added as the applied NH_4^+ should not significantly increase the inorganic N pool.

Soil samples are collected immediately following the application of $^{15}N\text{-}NH_4^+$ to determine the initial amount of $^{15}N^{14}N\text{-}NH_4^+$ and its isotopic signature. A second set of soil samples is collected at time "t" and again the amount of $^{15}N^{14}N\text{-}NH_4^+$ and its isotopic signature are determined. Although only two sets of samples are needed to determine the rate of gross mineralization, additional sampling intervals will improve estimates of mineralization rate.

Ammonium is extracted from the equivalent of 15 g dry soil with 75 mL 2 *M* KCl and shaken for 1 h. Pass the KCl extract through a Whatman No. 1 filter. Because filters can be contaminated with NH_4^+ or NO_3^-, they should be prewashed with the KCl solution [4].

The diffusion technique is widely used to concentrate inorganic N prior to determination of the isotopic signature of NH_4^+ and NO_3^- [5]. For both NO_3^- and NH_4^+, disposable specimen cups (120 mL) with airtight lids are often used. The NH_4^+ in the solution is converted into NH_3 by adding MgO, and an acid trap is floated in the solution. The acid trap is encased in Teflon® and consists of an N-free filter paper disk onto which 5 μL of 2.5 *M* $KHSO_4$ is pipetted. The MgO converts all the NH_4^+ in the solution to NH_3, which is subsequently trapped again in the filter paper. The entire procedure takes place at room temperature in about a 1 week period. Once all the NH_3 has been trapped, the filter paper can be analysed for total N and its isotopic composition. The diffusion method is also used to determine the isotopic composition of NO_3^-, and similar specimen containers are used. The NO_3^- in the solution is first converted into NH_4^+ by Devarda's alloy, and then the NH_4^+ is converted into NH_3 and trapped again in filter paper. Precautions have to be taken that all the NH_4^+ is removed from the solution before the conversion of NO_3^- to NH_4^+ takes place. The removal of NH_4^+ in the solution is achieved by increasing the pH to >13 by adding NaOH. Blank determinations are used to correct for the N contents of the reagents, particularly Devarda's alloy.

The gross rate of mineralization (m) is calculated as follows:

$$m = \frac{\left[NH_4^+\right]_0 - \left[NH_4^+\right]_t}{t} \times \frac{\log \frac{\text{atom \%}^{15}NE_0}{\text{atom \%}^{15}NE_t}}{\log \frac{\left[NH_4^+\right]_0}{\left[NH_4^+\right]_t}}$$

where

m	is gross N mineralization rate (mg N/kg soil·day),
t	is time (days),
$[NH_4^+]_0$	is total NH_4^+ concentration at time 0,
$[NH_4^+]_t$	is total NH_4^+ concentration at time t,

atom $\%^{15}NE_o$ is at.$\%^{15}$N excess of NH_4^+ at time 0,
atom $\%^{15}NE_t$ is at.$\%^{15}$N excess of NH_4^+ at time t.

Natural background enrichment in ^{15}N has to be determined before the at.$\%^{15}$N excess can be calculated. Most background ^{15}N abundance values for N-NH_4^+ are higher than 0.3663 at.%, the value measured for atmospheric N_2. If the natural background value of ^{15}N is unknown, a safe value to use would be 0.3700 at.$\%^{15}$N.

The amount of NH_4^+ consumption (C_a) is equal to:

$$C_a = m - \frac{\left[NH_4^+\right]_t - \left[NH_4^+\right]_0}{t}$$

where C_a is expressed in mg N/kg soil·day

An example to calculate gross mineralization (m) follows:

Assume you added 2 mg N/kg of ammonium sulfate at 99 at.% ^{15}N, with samples taken at t = 0 and t = 4 days.

Time (days)	NH_4^+ pool (mg N/kg)	NH_4^+ enrichment (at.% ^{15}N excess)
0	20	5
4	10	1

$$m = \frac{20-10}{4} \times \frac{\log 5/1}{\log 20/10} = 2.5 \times \frac{0.7}{0.3} = 5.8 \text{ mg N/kg·day}$$

The rate of NH_4^+ consumption is: $5.8 - \frac{10-20}{4} = 8.3$ mg N/kg soil·day

4.2.3. FACTORS AFFECTING NITROGEN MINERALIZATION

4.2.3.1. Characteristics of organic materials

Net N mineralization from organic matter, i.e. added organic material or soil organic matter, is predominately dependent on three interrelated factors: the quality of the organic matter, the physicochemical environment and the composition of the decomposing community [6]. Whereas the quality of the organic matter and the physicochemical environment can be manipulated to some extent, the composition of the decomposing community is largely a reflection of the first two factors. For example, an undisturbed forest soil is likely to have a higher abundance of fungi than an agricultural soil under conventional tillage. The high C:N ratio of the litter on a forest floor would lead to a higher colonization by fungi, whereas the incorporation of a legume cover crop with a low C:N ratio would stimulate the growth of chemo-heterotrophs rather than of a fungal population.

The quality of organic matter and how its mineralization is regulated has been interpreted in terms of the C:N ratio (Table I). It remains a widely used indicator of organic matter quality and has been used to predict the net conversion of organic N into mineral N. Because N controls the growth and the rate of turnover of soil microorganisms, it plays a key role in the

rate of decomposition of organic matter [6]. Whereas the theoretical optimum of the C:N ratio of the organic matter for microbial growth is approximately 25, organic matter like plant residue can show ratios between 10 and 1500. The %C in plant residue is around 45% and is a robust value independent of plant growth conditions. Therefore, the C:N ratio in the plant material is almost exclusively controlled by its N content and not its C content. Greenmanure legumes harvested before flowering can have an N concentration of 5%, equivalent to a C:N ratio of 9. On the other hand, woody material may contain less than 0.1% N, therefore a C:N ratio as high as 1500 is possible.

Although C:N is generally a good indicator of the quality of organic material and its rate of decomposition in soil, mineralization is affected also by the presence of polyphenols and lignin [7], high concentrations of which slow mineralization. Therefore, with high polyphenol or lignin content, the N:polyphenol or lignin:N ratio could be a better indicator of the rate of decomposition. Most annual crops like cereals and legumes, however, do not have elevated polyphenol or lignin concentrations, and the C:polyphenol or C:lignin ratio is not always a good predictor of the rate of decomposition.

TABLE I. CHEMICAL, PHYSICAL AND BIOLOGICAL ATTRIBUTES AND MANAGEMENT PRACTICES WHICH AFFECT THE RATE OF DECOMPOSITION OF ORGANIC MATERIALS

Parameter/management factor	Change in the rate of decomposition[a]
High %N in organic material	+
High %polyphenol in organic material	–
High %lignin in organic material	–
High C:N ratio	–
High polyphenol:N ratio	–
High lignin:N ratio	–
Increase soil moisture	+
Temperature (20–35°C)	+
Soil texture	+/–
High bulk density	–
Fertilizer addition	+/ no effect
Increased tillage/aeration	+
Increase nutrient availability	+
Incorporating organic material in the soil	+
Reducing size of the organic material[b]	+
Presence of soil fauna	+
Microbial diversity	?
Increased atmospheric CO_2 concentrations	?
Long term flooded conditions	–

[a] + or – indicates a likely increase or decrease in the rate of decomposition.

[b] For high C:N ratio material, net immobilization may occur in the short term.

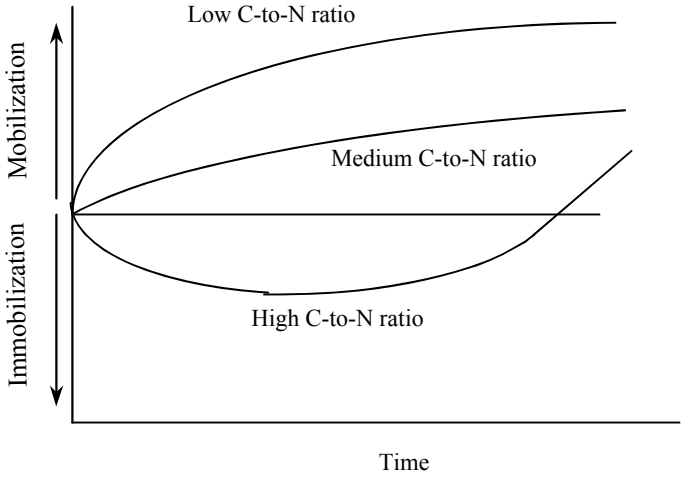

FIG. 3. Time course of mobilization and immobilization of nitrogen from residues as affected by their carbon-to-nitrogen ratio.

Depending on the quality of the organic matter, inorganic N will be immobilized or organic N will be released as inorganic N. If it has a high C:N ratio and lignin content, an initial period of N immobilization will occur, followed by a second period when N will be mobilized (Fig. 3). How long the first period of N immobilization will last and when the second period begins is dependent on the quality of the organic matter and the physicochemical environment under which the decomposition occurs. If C:N is close to 20 or lower, decomposition will be fast and NH_4^+ will be released almost immediately and converted into NO_3^- under aerobic conditions. If the temperature for microbial activity is at its optimum, the period of immobilization would be shorter, whereas a soil temperature of 10°C would prolong it.

Using ^{15}N labelled residue to follow the flow and fate of ^{15}N from organic into inorganic N forms is an elegant way to determine the release of N. A plant can be grown in an ^{15}N enriched solution. However, it is highly recommended to grow the labelled ^{15}N plant material in the field. Similar plant characteristics for glasshouse and field-grown plants are difficult to obtain, as growth conditions in a controlled environment and in the field are dissimilar. Differences in growth conditions affect residue quality. The chemical composition of the plant residue, e.g. C:N ratio and polyphenolic concentration, is dependent on the availability of inorganic N. As it is difficult to obtain similar concentrations of available N in the greenhouse and in the soil during the growth season, it is recommended to carry out the ^{15}N labelling process in the field rather than under controlled conditions. In particular, when unlabelled material is used in the field to obtain quantitative data such as yield biomass, residues should be generated under field conditions.

4.2.3.2. Determination of nitrogen mineralized using nitrogen-15 labelled residue

The amount of ^{15}N needed to label the plant material depends largely on (1) how much plant material will be applied, (2) for how long the ^{15}N from the residue will be traced in the mineral N pool and/or in the crop that has received the labelled residue, and (3) the size of the N pool in the soil (Table II). If a total budget of the ^{15}N residue is to be constructed, the size of the soil-N pool becomes particularly important, as a large enough amount of ^{15}N residue has to be added to obtain an ^{15}N value of the total soil-N pool, i.e. significantly enriched above its background value.

TABLE II. A SELECTION OF FIELD STUDIES USING IN-SITU NITROGEN-15 LABELLING TECHNIQUES TO FOLLOW THE FATE OF NITROGEN FROM RESIDUES IN SUBSEQUENT CROPS

Species labelled in the field with ^{15}N	Fertilizer N applied (kg/ha)	^{15}N excess (at.%)	N applied as residue (kg/ha)	^{15}N excess in residue (at.%)	Reference
Vicia dasycarpa	9	49	120	0.355	[8]
Oryza sativa	20	10	50	0.449	[9]
Desmodium ovalifolium[a]	4.3	99	316	0.990	[10]
Pueraria phaseoloides[a]	4.3	99	262	1.235	[10]
Glycine max	75	10	150	1.780	[11]
Phaseolus vulgaris	75	10	150	0.840	[11]
G. max	168	5	32	1.142	[12]
Arachis hypogaea	10	10	100–130	0.137–0.164	[13]
V. villosa[b]	120–130	0.01	107–115	0.230–0.275	[14]
Pisum sativum[c]	15.6	10	96	0.237	[15]

[a] Foliar labelling with ^{15}N urea.

[b] ^{15}N depleted $(NH_4)_2SO_4$.

[c] Landscape study with 100 ^{15}N microplots; ^{15}N enrichments in the residues ranged between 0.0617 and 0.4677 at.% ^{15}N excess; amounts of N applied as residue were between 23.4 and 276 kg/ha.

Most field studies on the release of N from residues are carried out for one growth season only, as (1) the enrichment required to follow the flow of the labelled ^{15}N residue for more than 1 year becomes too high and its associated cost prohibitive, and (2) the integrity of ^{15}N microplots can often not be guaranteed. The amounts of residue ^{15}N applied and their ^{15}N enrichments have varied widely (Table II). In these studies, the residue was labelled in the field.

In the field, plants should be grown in ^{15}N labelled microplots. To avoid exchange of N between the microplot and the soil outside, a buried enclosure or barrier can be used to contain the ^{15}N within the microplot and exclude root access to unlabelled soil at the microplot margin. If no borders are used, the plants close to the edge of the microplots will be less enriched than those growing in the centre of the microplot. However, the ^{15}N labelled residue in the entire ^{15}N microplot can be used to follow the uptake of ^{15}N in the subsequent crop as long as all the residues are mixed thoroughly before application. The overall enrichment of the plants grown in a ^{15}N microplot without a border will be lower than the enrichment of the plants grown in a ^{15}N microplot with a border. As a precaution, several subsamples of the residue should be analysed for ^{15}N prior to the application, to ensure that the material is uniformly labelled [16].

The percentage N in the crop derived from the ^{15}N labelled residue (%Ndf^{15}NR) is equal to:

$$\%\text{Ndf}^{15}\text{NR} = \frac{\text{atom}\,\%\ ^{15}\text{N excess}_{\text{crop}}}{\text{atom}\,\%\ ^{15}\text{N excess}_{\text{residue applied}}} \times 100$$

4.2.3.3 Determination of nitrogen mineralized using unlabelled residue and nitrogen-15 labelled fertilizer

An indirect ^{15}N method has also been used to estimate the amount of N from residues that is accumulated by a subsequent crop. Nitrogen-15 labelled fertilizer is applied to the soil, and unlabelled legume or non-legume residues are incorporated (see Ref. [17]). The ^{15}N enrichments in crops grown in the presence and absence of residues are determined. The dilution of the ^{15}N in the crop grown in the presence of the residue compared with the ^{15}N enrichment in the crop grown without residue is the contribution of the residue N to the crop [12, 18].

The percentage N in the crop derived from unlabelled residue (%NdR) is equal to:

$$\%\text{NdfR} = \left(1 - \frac{\text{atom}\%\ ^{15}\text{N excess}_{\text{+residues}}}{\text{atom}\%\ ^{15}\text{N excess}_{\text{-residues}}}\right) \times 100$$

When the direct and the indirect ^{15}N methods were compared across a landscape, the contribution of legume residue N to subsequent wheat was apparently greater with the latter [15]. Added ^{15}N interactions may play a role when the rate of decomposition of organic matter is affected differently when ^{15}N fertilizer or ^{15}N labelled residue is applied. However, Hood [11] found in another field study that both methods provided close estimates of the supply of legume N to a following maize crop. As the indirect method has not been used extensively under field conditions, it may be too early to judge its accuracy in estimating contributions of legume N to subsequent crops. If reliable under a wide range of field conditions, the indirect method would be a simple and convenient way to determine the contributions of residue N to subsequent crops.

4.2.3.4. Soil characteristics

Whereas decomposition of organic amendments occurs on the soil surface (residue mulch) or even without any contact with the soil, there is general agreement that incorporation increases decomposition. The increase in decomposition is likely to be caused by improvement of the microclimate, which promotes decomposition. Residues left on the surface often dry out faster than incorporated residues, thereby impairing decomposition.

Although soil texture may have a limited role in the initial decomposition of added residue, it can exert strong control on the total amount of organic material that is eventually protected from decomposition. In contrast to sand and silt particles, clay particles provide better protection for organic material against microbial activity. Along similar lines, the formation of soil aggregates with inclusion of organic materials serves as a further protection against decomposition.

Soil fauna, i.e. earthworms, millipedes, protozoa, termites and nematodes, increase N mineralization because they:

— produce enzymes that are involved in the disintegration of complex and large molecules into smaller compounds,
— improve the biophysical environment of heterotrophs, and
— reduce the size of the organic material, thereby increasing the surface area.

4.2.3.5. Environmental factors

Temperature and moisture are the two environmental factors that have the strongest impact on the rate of decomposition of organic material. Whereas decomposition will take place at low temperatures, its optimum rate occurs at around 25–30°C. One reason that tropical soils often have lower organic matter contents than soils in temperate zones is that the soil temperature in the tropics is near optimum. Although inputs of organic residues may be similar under tropical and temperate conditions, the rate of decomposition is higher in the tropics [19].

As water is needed for all basic biological processes, moisture has a strong influence on metabolic processes in the soil. Whereas little or no decomposition will take place in a dry soil, decomposition will continue in a flooded soil. The net increase in NH_4^+ during incubation of soil under waterlogged conditions is used as one indicator of the amount of potentially available N [20]. However, anaerobic decomposition is energetically less efficient than is aerobic decomposition. Prolonged flooded conditions in intensive rice cropping systems in Asia appear to have caused changes in the chemical properties of organic matter, leading to lower N availability despite higher total soil-N content [21]. Although the early stages of the decomposition processes remain largely similar under aerobic and anaerobic conditions, the end products under aerobic conditions are CO_2 and H_2O, whereas under waterlogged, anaerobic conditions they are CO_2, H_2O and CH_4.

The atmospheric CO_2 concentration is increasing at a rate of 1.8 ppm per year. Although increased CO_2 concentrations can lead to higher photosynthesis and increased biomass production, the quality of residue produced under elevated CO_2 may decline. A general increase in the C:N ratio of the residue may occur, causing an increase in the rate of immobilization and a possible lower rate of decomposition. However, few long term studies ($\geq$10 years) have been conducted on the impact of elevated CO_2 on the quality of residue and its subsequent impact on mineralization. Once a new (pseudo-)steady state has been reached for the plant–soil system, not only will the quality of the plant material have changed, it is also likely that the soil microbial population will adjust accordingly, leading to no significant changes in the rate of mineralization.

4.2.3.6. Management factors affecting mineralization

Intensive tillage affects the rate of mineralization of organic matter and plant residues [22]. Two major factors cause increases in decomposition following soil disturbance: (1) an increase in the O_2 concentration in the soil profile, which in turn promotes decomposition, and (2) the loss of physical protection of the C from microbial degradation because of a breakdown of aggregates. Soil temperature and soil moisture content are also altered by soil disturbance, but their combined net effect on the rate of decomposition is less than the effects of increased aeration and loss of physical protection.

Incorporation of residues into the soil increases the rate of decomposition. Lower nutrient availability for microbial activity and drier conditions when residues remain on the surface are the likely causes for lower rates of mineralization. Increased nutrient availability through fertilization leads to higher inputs of nutrients from residues, but may also improve residue quality and nutrient availability, leading to increased mineralization.

Combining high quality (low C:N) with lower quality (high C:N) residues is a management practice to control the rate of net N mineralization. By combining *Sesbania* and rice residues with a wide difference in their C:N ratios, the release of N from the residues was better synchronized with the predicted daily rate of N uptake by rice [23]. Such improved synchronization of net N mineralization and crop-N demand led to lower N losses and higher residue N use efficiency. The challenge lies in choosing residues of differing qualities such that the rate of net N mineralization matches the rate of N uptake by the crop. However, a number of biotic and abiotic factors that influence the rate of decomposition of residue and the daily rate of N uptake by the crop are outside the control of the farmer (soil and air temperature, soil moisture in non-irrigated environments, solar radiation). Therefore, it will remain a challenge for the farmer to manipulate the overall quality of residues through mixing to synchronize the release of N with the demand for N by the crop.

4.2.4. LOSSES OF NITROGEN FROM SOILS AMENDED WITH ORGANIC MATERIALS

4.2.4.1. Denitrification

Denitrification is an anaerobic process in which NO_3^- is converted into N_2O and subsequently into N_2. Most denitrifying microorganisms are facultative denitrifiers; under aerobic conditions they use O_2 as the electron acceptor and produce CO_2, whereas under O_2 limiting conditions NO_3^- is used as the electron acceptor and N_2O and N_2 are produced. Not all of the N_2O produced is further reduced to N_2. The ratio of N_2O:N_2 produced depends on the availability of water-soluble C, temperature, concentration of NO_3^- and pH [24].

Losses of N via denitrification vary widely across a field, as denitrification is a highly variable process [25, 26]. Key regulatory abiotic factors controlling denitrification are soil water content, soluble organic C, and NO_3^-availability [24].

Denitrification losses from organic material can be determined by measuring release of N_2O and N_2. However, because the amount of N_2 in the atmosphere is high (79%) the N_2 balance of the headspace in a container cannot be used to estimate denitrification because of insufficient precision. In contrast, the background concentration of N_2O in the atmosphere is low (<0.5 ppm) and the relatively large change in N_2O concentration can be measured easily by gas chromatography. By blocking the conversion of N_2O to N_2 by adding C_2H_2 (10% by volume), N_2O becomes the sole end product of denitrification. If a particular organic material were the sole source of NO_3^-, the N_2O produced in the presence of C_2H_2 would equal the loss of N from the residue. However, in a soil it is unlikely that all of the N_2O produced came from added organic material.

An incubation chamber (1 L) can be installed on top of the ^{15}N microplot, C_2H_2 (10% by volume) added and the gas phase analysed for ^{15}N-N_2O at time 0 and 4 h. Depending on the rate of denitrification, a shorter or longer incubation period can be used. Gas samples can be taken from the incubation chamber through a gas sampling port using a syringe (15 mL), stored in pre-evacuated sealed containers, and subsequently analysed for ^{15}N composition by

an isotope ratio mass spectrometer (IRMS). The amount of N_2O in the gas phase should be determined independently from the IRMS, using a gas chromatograph equipped with an electron capture detector. The rate of N_2O produced can be calculated from the incremental increase in N_2O during the 4 h incubation period. Procedures for the determination and isotope ratio analysis of N_2 and N_20 were given by Stevens et al. [27, 28].

Another approach is to take two soil samples from the centre of the ^{15}N microplot using sampling tubes (10 cm long, 5 cm in diameter), place the tubes in an incubation jar (1 L) provided with a sampling port/stopper, replace 10% of the air with C_2H_2 and analyse the gas phase for ^{15}N-N_2O at time 0 and 60 min. The jars should be incubated at a temperature that is similar to the soil temperature in the field.

Difficulties with the chamber and the incubation jar method are that C_2H_2 does not stop denitrification completely and a portion of the N from the residue is emitted as N_2 rather than N_2O. In particular, when an incubation chamber is used under field conditions, the C_2H_2 may not diffuse deeply enough into the soil profile to block conversion of N_2O into N_2.

An example of the calculation of the amount of residue N lost via denitrification follows:

— Amount of ^{15}N residue added: 10 g/m^2;
— ^{15}N enrichment of residue applied: 25.5255 at.% ^{15}N excess;
— Amount of N_2O at time zero: 0.25 ppm at 0.3688 at.% ^{15}N;Amount of N_2O at 60 min: 5.88 ppm at 5.2555 at.% ^{15}N;
— Size of the incubation chamber: 1.25 L.

Calculation:

— The change in N_2O production is 5.88 – 0.25 ppm = 5.63 ppm.
— 1 ppm is equal to 1 μL/L.
— The volume of N_2O produced is 5.63 × 1.25 = 7.038 μL.
— The number of moles of N_2O produced is $\frac{7.038}{22.4 \times \frac{298}{273}} = 0.288$ μmol
(Comment: the volume of 1 μmol of N_2O is 22.4 μL at standard temperature and pressure.)
— The mass of N produced as N_2O is 0.288 × 2 × 14.053 = 8.089 μg/chamber·h. (Comment: The atomic weight of 1 mol of N at 5.2555 at.% ^{15}N is 14.053 g.)
— The fraction of N derived from the residue is $\frac{5.2555 - 0.3688}{25.5255} = 0.191$.
— The mass of N derived from the residue is 0.191 × 8.089 = 1.549 μg N/chamber·h.

4.2.4.2. Leaching

Once organic N is mineralized and converted into NH_4^+ and subsequently into NO_3^-, it can be lost via leaching. Whereas the movement of NH_4^+ in the soil is limited by bonding to the cation exchange complex (CEC) of clay particles and soil organic matter, NO_3^- remains in solution and susceptible to leaching. Leaching is here defined as N that has moved downward in the soil profile below the rooting zone. Overfertilization, when the supply of N far exceeds the demand for N by the crop, can lead to large N leaching losses. Leaching losses often occur when soil-N mineralization takes place or when organic N is applied in the form of manure during a fallow period, e.g. during the winter months in the northern hemisphere. During those months, leaching losses are often accentuated by an abundance of precipitation

combined with low evapotranspiration losses, which enhance water saturation, leading to subsurface or surface water flow. During the summer months, leaching losses are usually low; evapotranspiration exceeds precipitation and water use by crops is high.

Leaching loss of N from organic material depends mainly on two factors. Foremost is the net amount of organic N that is mineralized and converted into NO_3^-. Secondly, once the organic N is nitrified to NO_3^-, sufficient water has to be present to allow downward movement [29]. However, if there is too much water, the soil becomes waterlogged and NO_3^- will be lost via denitrification. Soil texture is an important property that determines how much NO_3^- is lost via leaching or denitrification. A clay soil with a heavy texture has a lower hydraulic conductivity than a lighter, sandy soil. Therefore, in a heavy textured soil, NO_3^- is more susceptible to denitrification whereas in a sandy soil, leaching is more likely to be the prime mechanism of NO_3^- loss.

To quantify the amount of N lost from organic material through leaching, ^{15}N labelled organic material is applied to the soil and ^{15}N is measured below the rooting zone. The most common method to collect leached NO_3^- below the rooting zone is to install sampling probes or ceramic cups (suction lysimeters) to collect the soil solution for analysis of total NO_3^- content and its ^{15}N signature by standard techniques [5]. Sampling probes should be of sufficient length for collection of solutes below the rooting zone. As water availability is a key factor that controls rooting depth, longer sampling probes are usually needed in dryland systems than in irrigated systems. If there is evidence that, in addition to NO_3^-, organic ^{15}N has also leached below the rooting zone, the solutes should be analysed for both organic and inorganic N content and its isotopic signature. To calculate the total amount of ^{15}N lost during a growing season or winter months, frequent sampling is necessary.

4.2.4.3. Volatilization

Losses of N via volatilization of NH_3 from organic residues can be significant. Ammonia volatilization losses were between 37 and 170 kg N/ha (mean of 82 kg N/ha) when the N was applied as urine, and when applied as dung, losses were between 2 and 156 kg N/ha with a mean of 48 kg N/ha. In a grazed area of legumes/pastures, losses ranged from 1 to 17 kg N/ha with a mean of 7 kg N/ha [30]. In a controlled study, after 95 days, 8% of surface applied alfalfa N was lost as NH_3, which increased to 12% when the alfalfa was treated with a herbicide [31]. Clearly, when a total ^{15}N budget is to be constructed, losses due to volatilization should not be ignored. Unfortunately, measuring volatilization losses from residues under field conditions remains a challenge because of the complexity of the system and the infrastructure that is needed to measure NH_3 loss.

Volatilization losses of NH_3 can be measured in the field by micrometeorological techniques or wind tunnels. Micrometeorological techniques are considered to be the most accurate of the methods available. A disadvantage is that they require a circular area with a radius of 15–40 m. Such a large area makes this method unsuitable for quantification of ^{15}N volatilization losses from ^{15}N labelled organic material. Another disadvantage is the need for special instrumentation such as a wind-speed meter (cup anemometer), air temperature probes, data loggers, NH_3 collection samplers and the expertise to operate the equipment.

Another approach is to use a closed chamber. Losses of NH_3 are dependent on environmental factors: temperature, precipitation and wind-speed. An increase in wind-speed led to as much as 16 times higher volatilization losses when measured by the micrometerological method than when measured by the chamber method [32]. As NH_3 is reactive with water, an elevated

NH_3 concentration in a static chamber will lead to a reduction in the NH_3 emission and hence an underestimation of loss.

Following the application of ^{15}N labelled material, a chamber (20 cm in diameter and 10 cm deep) can be pushed 2–3 cm into the soil where the material is applied. The chamber is equipped with inlet and outlet ports and hooked up to a vacuum pump. The rate of the airflow through the headspace is controlled and the NH_3 in the gas stream collected in an acid trap [33]. A solution of 0.05 *M* H_2SO_4 (650 mL) or 0.002 mol/L H_3PO_4 (120 mL) has been used as acid traps [33, 34]. Once the NH_3 has been trapped and converted into NH_4^+, its concentration can be measured by the standard diffusion method and its isotopic signature determined [5]. As the time period during which NH_3 was collected is known, the rate of N loss via volatilization from the organic material can be calculated.

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4.3. CONTRIBUTIONS OF ORGANIC SOURCES TO PLANT NUTRITION

B. VANLAUWE
Tropical Soil Biology and Fertility Institute of CIAT,
Nairobi,
Kenya

A historical perspective of organic matter management in agriculture is provided, and factors affecting organic residue decomposition in soil are discussed in this session. The relationship between organic resource quality and nitrogen release for plant uptake, and potential contributions of organic inputs to soil fertility and crop yields are also discussed, with particular emphasis on tropical agriculture.

4.3.1. INTRODUCTION

Organic resources play a critical role in maintaining fertility of tropical soils and are an integral part of the currently adopted integrated soil fertility management (ISFM) research and development paradigm for tropical soil fertility research [1, 2]. Unlike mineral fertilizers, the release dynamics of plant available N from organic resources vary widely and are less predictable. Such uncertainties may hinder the most efficient use of these organic resources or even their adoption by small scale farmers.

4.3.1.1. A short history of the science of organic matter management

Although organic inputs had not been new to tropical agriculture, the first seminal synthesis on organic matter management and decomposition was written only in 1979 by Swift et al. [3] (Table I). Between 1984 and 1986, a set of hypotheses was formulated based on two broad themes, "synchrony" and "soil organic matter" (SOM) [4–6], building on the concepts and principles formulated in 1979. Under the first theme, the O(rganisms)-P(hysical environment)-Q(uality) framework for OM decomposition and nutrient release [3], formulated earlier, was worked out and translated into hypotheses driving management options to improve nutrient acquisition and crop growth. Under the second theme, the role of OM in the formation of functional SOM fractions was stressed. During the 1990s, the formulation of the research hypotheses related to residue quality and N release led to a vast number of projects aimed at validating these hypotheses.

Two major events further accentuated the relevance of the topic in tropical soil fertility management. Firstly, a workshop held in 1995 with the theme "Plant Litter Quality and Decomposition" resulted in a book summarizing the state of the art of the topic [7]. Secondly, CIAT's Tropical Soil Biology and Fertility (TSBF) Institute, in collaboration with its national partners and Wye College, developed the Organic Resource Database (ORD) and related Decision Support System (DSS) for OM management (Fig. 1) [8]. Careful analysis of the information contained in the ORD (see below) led to the development of the DSS, which makes practical recommendations for appropriate use of organic materials, based on their N, polyphenol and lignin contents, resulting in four categories of materials (Fig. 2).

TABLE I. SUMMARY OF THE SCIENCE OF TROPICAL ORGANIC RESOURCE MANAGEMENT

Period	Observation	Reference
<1970s	Organic matter as a "blob"	C.A. Palm (personal communication)
1979	Organisms – Physical environment – Quality framework for organic matter decomposition	[3]
1984–1986	Development of the "synchrony" research theme within the Tropical Soil Biology and Fertility programme	[4–6]
1990s	Various experiments addressing the "synchrony" hypothesis	Various
1995	International Symposium on "Plant Litter Quality and Decomposition"	[7]
2000	Development of the 'Organic Resource Database' and the Decision Support System for organic N management	[8]
>2001	Quantification of the Decision Support System for organic N management	Various

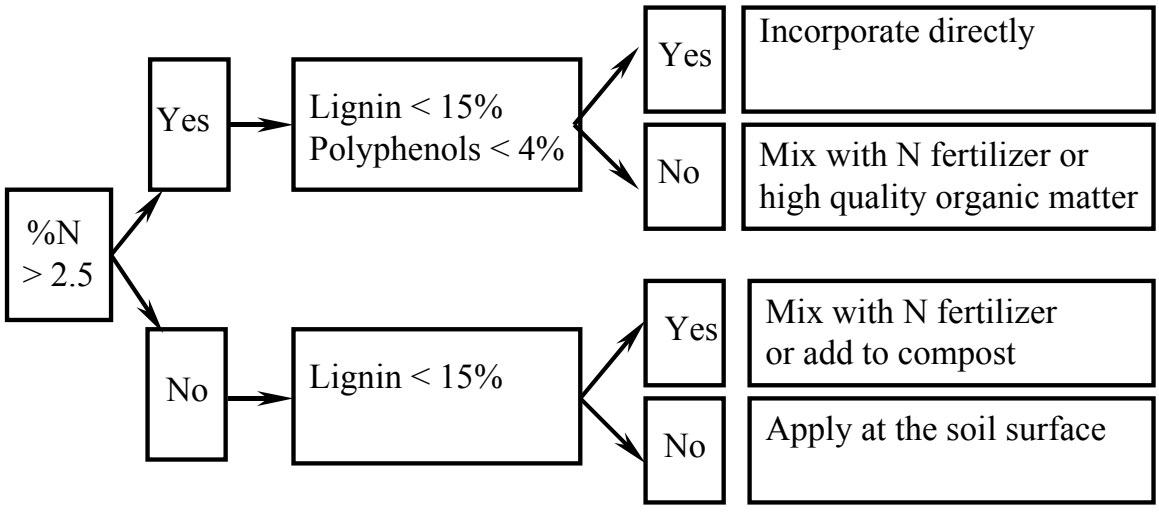

FIG. 1. The Decision Support System for organic matter management [8].

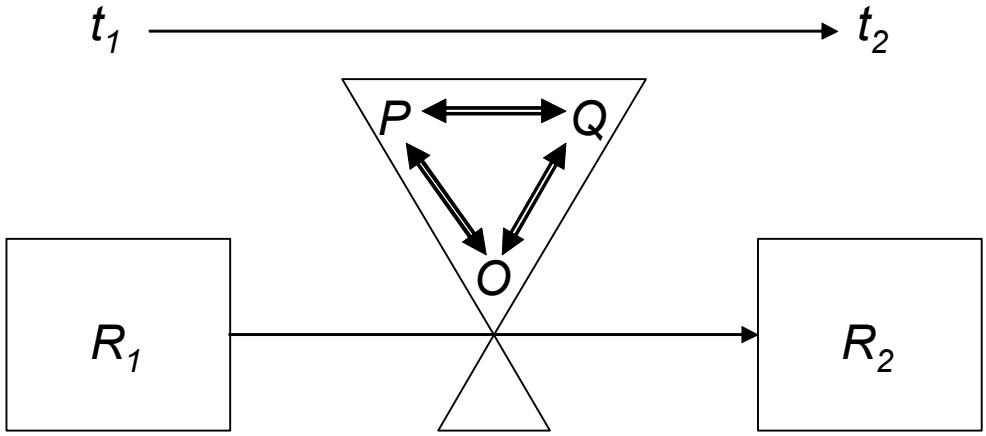

FIG. 2. Diagrammatic presentation of the decomposition process resulting in the change of an organic resource from state R_1 to R_2 between time t_1 and t_2. The rate of change is regulated by three interacting factors:, the physicochemical environment (P) and resource quality (Q) acting through decomposer organisms (O) [8].

4.3.2. ORGANIC RESIDUE DECOMPOSITION

4.3.2.1. O(rganisms)-P(hysical environment)-Q(uality) framework

Swift et al. [3] proposed a framework in which the basic module represents any organic resource, which is changed from state R_1 to R_2 over time (t_1 to t_2) (Fig. 2). The module is repeated in the form of a cascade in which the products of decomposition of one resource become the initial resources for subsequent modules in the cascade. The rate of change from R_1 to R_2 is regulated by a combination of three interacting groups of factors: the physicochemical environment (P) and the quality of the resource (Q), acting through the organisms constituting the decomposer community (O) (Fig. 2). Decomposition of any resource is the result of three component processes:

— catabolism, i.e. chemical changes such as mineralization, giving rise to inorganic forms and the synthesis of decomposer tissues and humus;
— comminution, by which there is a physical reduction in particle size and often selective redistribution of chemically unchanged litter; and
— leaching, which causes transport down the profile or removal from the system of labile resources in either changed or unchanged form [9].

Various soil organisms (the “O” factor) contribute directly or indirectly to the decomposition and mineralization processes. While microorganisms are directly responsible for most of the OM breakdown and mineralization, soil fauna greatly influence the decomposer flora as a result of their feeding activities. Fauna also influence decomposition by litter comminution and a consequent increase in surface area of the substrate. While certain processes require specific (micro)organisms, e.g. nitrification requires the presence of *Nitrosomonas* spp. and *Nitrobacter* spp., other processes can be catalysed by a wider range of organisms. Most evidence to date, however, shows a rather high level of redundancy for maintenance of specific decomposition processes [10].

The physicochemical environment (the "P" factor) comprises soil and climate conditions and affects both the quality of organic resources and the decomposition process itself through regulation of microbial and faunal activity. Soil moisture provides water for tissue growth and a medium for enzyme activity and activity of organisms such as nematodes and protozoa. The response of all enzyme catalysed reactions is sensitive to temperature and follows the Arrhenius equation, which predicts that the logarithm of the reaction rate will be linear with the reciprocal of the absolute temperature [11]. Response to temperature is often expressed by the temperature coefficient Q_{10}, the fold increase in response to a 10°C increase in temperature, and often assumed to approximate 2 for biological systems. The adaptation of various organisms to temperature also differs widely. The highest level of decomposition activity is to be found in aerobic environments, as anaerobiosis usually results in incomplete degradation of substrates and the accumulation of OM. Oxygenation in soil crumbs may be limited due to the slow rate of diffusion of oxygen in water, resulting in anaerobic spots, even in well aerated soils. Bacteria tolerate relatively narrow ranges of pH at the alkaline end of the spectrum, in contrast to fungi, which have generally broad optima but are most active at acid pH. The latter seem able to maintain their internal pH between 5 and 6, as for most invertebrates [3]. The soil environment also indirectly affects decomposition through adsorbance of fresh organic substrates on inorganic soil constituents such as clays, silt and metal sesquioxides [9]. Generally, while climate sets upper and lower limits to the potential decay rate, the fine-tuning at local levels is determined by resource quality and the edaphic complex [3].

The O, P, and Q factors interact with each other in many other ways and, consequently, indirectly affect the decomposition/N mineralization of organic resources. For instance, organic resource quality can be affected by nutrient status and pH of the soil and by increases in defence components such as polyphenols in the presence of metabolic resources in excess of growth demands [9]. Such defence compounds can be carried over to the litter after senescence, especially in the case of immobile defence compounds such as tannins and lignins, which are energetically expensive to construct [12]. Organic resource quality affects the composition of the decomposer community [13] and the soil physicochemical environment by providing extended soil surface cover for materials of lower quality and thus of lower decomposition rate.

4.3.2.1.1. Quality of the organic resource

A range of quality characteristics has been found to affect the decomposition and mineralization processes of organic resources. Originally, the C:N ratio was seen as a good predictor of decomposition and N availability [14]. Nitrogen is one of the commonest factors limiting litter decomposition, as it determines the growth and turnover of the microbial biomass mineralizing the organic C components. Organic materials with a C:N ratio above 20 usually result in immobilization of soil N due to lack of N in the decomposing resources. Mellilo et al. [15] showed that the lignin content of hardwood leaf litter residues significantly affected their decomposition, as lignin is one of the most recalcitrant naturally produce polymers. Vallis and Jones [16] reported that soluble polyphenols affect N mineralization dynamics of organic residues. This happens mainly through complexation of proteins by polyphenols, thus protecting these proteins from decomposition. Handayanto et al. [17] showed that the content of soluble polyphenols that were actively binding proteins was more closely related to decomposition than was the total soluble polyphenol content.

The superiority of certain indices over others for predicting N mineralization has perhaps been more related to the types of organic material being studied (crop residues, temperate tree-leaf

litter, or fresh leaves), or to the range of resource quality, rather than to real differences in the controls on decomposition and N release patterns. For instance, when looking at cereal residues, soluble polyphenols are unlikely to be included in quality–mineralization relationships, as such resources usually contain less than 10 g/kg polyphenols. Green manure residues, on the other hand, often contain substantial amounts of soluble polyphenols besides N, resulting in the former being an important modifier of the mineralization process [18].

Based on information available in the literature and their own analyses, Palm et al. [9] compiled the Organic Resource Database (ORD), which currently contains information on quality parameters of plant materials, including macronutrient, lignin and polyphenol contents of fresh leaves, litter, stems and/or roots for almost 300 species found in tropical agro-ecosystems (ftp://iserver.ciat.cgiar.org/webciat/ORD/). Following analysis of a substantial number of laboratory based N mineralization studies using a wide range of organic resources, Palm et al. [8] proposed a conceptual Decision Support System (DSS) for organic-N management (Fig. 1). Their DSS proposed four classes of organic resources, each having different N release dynamics. Class I materials have high N (>25 g/kg), low soluble polyphenol (<40 g/kg) and a low lignin (<150 g/kg) contents, and release their N quickly without initial N immobilization. Classes II and III materials have either high N (>25 g/kg) and high polyphenol (>40 g/kg) or high lignin contents (>150 g/kg) (class II), or low N (<25 g/kg), low polyphenol (<40 g/kg) and low lignin contents (<150 g/kg) (class III), resulting in an initial N immobilization phase. Class IV materials have low N (<25 g/kg) and high lignin contents (>150 g/kg), resulting in delayed decomposition. Organic resources, which rapidly release their N, are often referred to as high quality, whereas other residues with delayed N release and sometimes temporary immobilization of N are often referred to as low quality organic resources.

Farmyard manure, a commonly used organic resource, usually does not respect the above rules of thumb, probably because it has gone through a decomposition phase when passing through a digestive system [19] and because it is often stored, in various ways, before application to the field. Based on the above conditions, manure quality can vary considerably; pit stored manure usually has a higher N content than heap stored manure [20]. To complicate matters further, manure is often mixed with other organic inputs or household ash, resulting in differences in N content, e.g. from 0.35 to 2.47% in the West African sahel [21].

4.3.2.1.2. Organic resource quality determination

Various methods have been used to assess organic resource quality. While the choice of methodology may have little impact on the measurement of total C or N, this is certainly not true for assessing soluble polyphenol and lignin contents. Assessment of the total soluble polyphenol content is usually done using (a modification of) the method proposed by King and Heath [22]. For measuring these components, care is needed to fully standardize plant material preparation and the assay itself [23, 24]; the temperature for drying the plant materials [25] and the dry matter:extractant ratio strongly affect the final soluble polyphenol content [23]. Other approaches look more specifically at certain classes of polyphenols with varying potential effects on N release dynamics. Handayanto et al. [17] modified the assay to measure the protein binding capacity of soluble polyphenols. Other assays focus on measuring hydrolysable tannins [26] or condensed tannins [27]. Determination of lignin is most often achieved using the Van Soest assay; two routes can be followed [28, 29]. One assay dissolves all cell wall components except lignin with sulphuric acid, after an extraction with neutral detergent [28], whereas the other oxidizes the lignin with permanganate after an extraction with acid detergent fibre [29]. A detailed fractionation scheme for organic resource

quality assessment has been proposed by Palm and Rowland [24] (Fig. 3), although modifications of this scheme are possible.

4.3.3. ASSESSING NITROGEN RELEASE AND RELATIONSHIPS WITH ORGANIC RESOURCE QUALITY

Various approaches exist to measure the N release dynamics of organic resources. While certain methods measure the amount of N mineralized from organic resources, other approaches measure the amount of N remaining after a certain period of decomposition. Very often, results from these assessments are related to various organic-resource quality parameters using single or multiple regression analysis.

4.3.3.1. Laboratory incubation techniques

Direct assessment of the amount of mineral N produced usually happens in laboratory assays under controlled soil moisture and temperature conditions. One technology uses the aerobic incubation approach, in which organic materials are mixed with soil and the amount of mineral N produced is measured relative to a control soil without addition of organic materials (e.g., Ref. [18]). Another technique is based on a leaching assay, first used by Stanford and Smith [30], in which organic resources of interest are mixed with soil and incubated in tubes which are leached periodically with a N-free solution of cations and anions. The mineral N concentration in the leachate can then be estimated and the amount of mineral N removed at each sampling time calculated. In both cases, mineralization is measured at temperatures usually ranging from 25 to 35°C. The amount of organic residue to be added in each treatment may be calculated to give either equal amounts of dry matter or equal amounts of N (or other nutrients), depending on the objective of the experiment. As a rough guide, around 100 mg N/kg soil is a reasonable amount to add.

4.3.3.1.1. Litterbag techniques

The litterbag technique, in which a certain amount of organic resource is confined in a bag with a certain mesh size and either placed on the soil surface or incorporated (e.g., Ref. [31]), measures the amount of organic N remaining after a certain period of time. Decomposition and N release data are frequently modelled using a negative exponential equation [32]. With this technique, the decomposition process takes place under field conditions with reduced control of the environmental conditions relative to the incubation approaches. Furthermore, confinement in bags may affect the decomposition process through changes in microclimate conditions, reduced infiltration of rainfall or reduced litter/soil contact. Litterbag data also assume that N not recovered in the bags is available, whereas this N may have been incorporated directly into the particulate OM fraction.

4.3.3.1.2. Direct-labelling approaches

Finally, unconfined approaches usually use ^{15}N labelled organic resources and measure the amount of applied OM N retrieved in specific SOM fractions with varying turnover times (e.g., Ref. [33]). This approach, although more costly and tedious, gives the most realistic data regarding N release from organic resources and allows quantification of the fate of applied organic N in the crop, soil and soil solution.

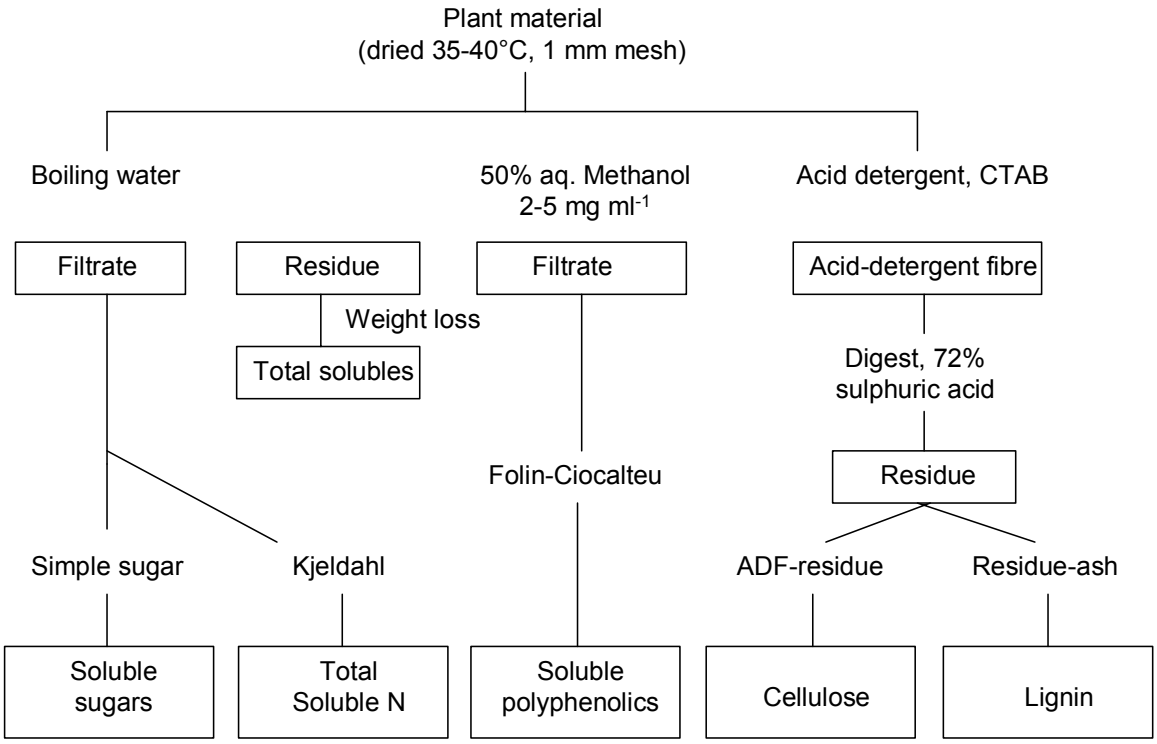

FIG. 3. Recommended procedures for analysis of carbon proximate fractions using three separate extractions [24].

4.3.4. CONTRIBUTION OF ORGANIC RESOURCES TO YIELDS AND SOIL FERTILITY

Nitrogen in organic materials is gradually mineralized after application to the soil, and part of that plant available N is taken up by crops, immobilized in the SOM pool, or lost in gaseous form or through leaching. Release of a substantial amount of N from medium to low quality organic resources often continues beyond a growth season, potentially resulting in significant residual effects.

4.3.4.1. Impact on yield

Short term data reveal a wide range of increases in maize grain yield in systems with added organic matter compared to the control systems (Fig. 4). With higher soil fertility status, the maximum increases were observed to decrease to virtually nil at control grain yields of about 3000 kg/ha. Although yields on fields with a low soil fertility status, e.g. with control yields below 1000 kg/ha, can easily be increased up to 140% by incorporation of a source of OM in the cropping system, this would lead to absolute yields hardly exceeding 1500 kg/ha (Fig. 4). In most cropping systems, absolute yield increases in the organic matter based treatments are far below 1000 kg/ha. Increases in maize grain yield at a certain control grain yield are, of course, quite variable and depend on a series of factors not considered in the graph, such as genotype, quality of the applied organic matter, general soil fertility status, organic matter management and agronomic practices.

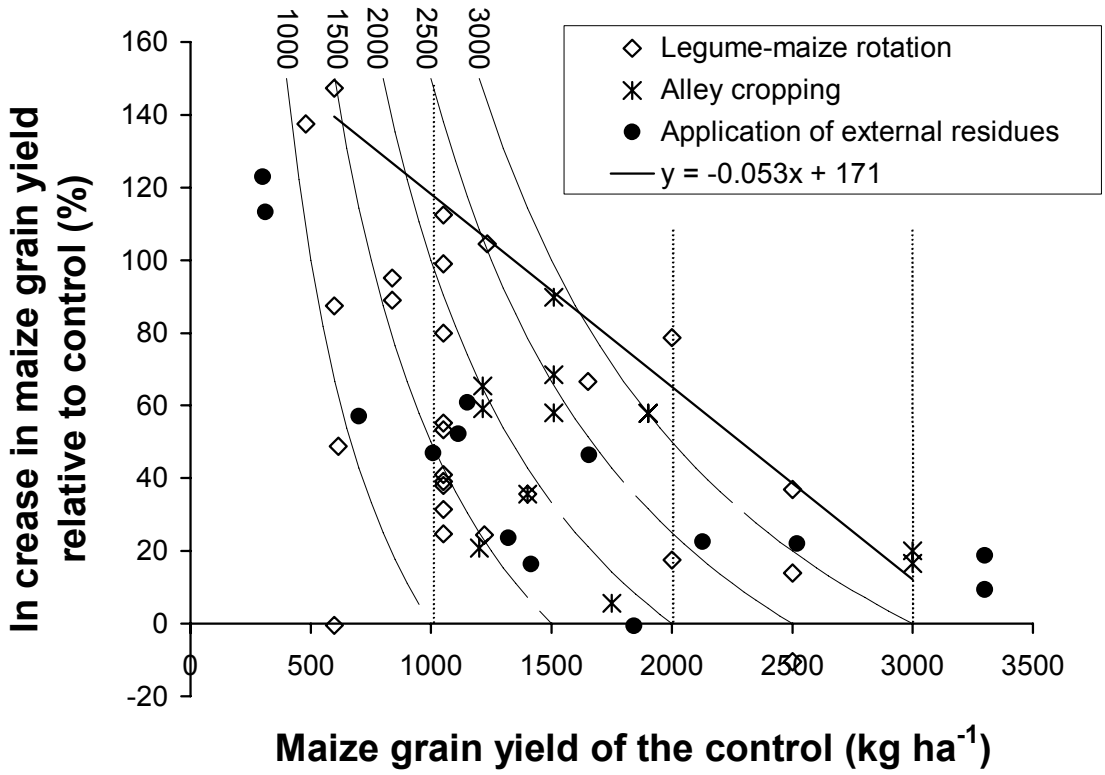

FIG. 4. Increase in maize grain yield relative to the control in cropping systems based on organic matter management (legume/maize rotation, alley cropping, systems with application of external organic matter) without inputs of fertilizer nitrogen as influenced by the initial soil fertility status, expressed as yield in the control plots. The linear regression line shows the estimated maximal increases in grain yield. The curved lines show the absolute yields in the treatments receiving organic matter (in kg/ha) [34].

The impact of applied organic N on crop yield is often expressed as its N fertilizer-equivalency (FE) value, calculated as the amount of N fertilizer applied to reach the same yield as the organic matter treatment (Fig. 5). Percentage N fertilizer equivalency (%FE) values are calculated as:

$$\%FE = \frac{\text{FE (kg N/ha)}}{\text{N applied as organic matter (kg N/ha)}} \times 100$$

In Fig. 5, FE values range from 52 to 77 kg N/ha while %FE values range between 87 and 129%. Values over 100% indicate that either the N released from the organic resource was used more efficiently by a crop, possibly because of improved synchrony between supply of, and demand for, N, or other growth limiting factors besides N were alleviated by applying the organic resource. Trials aimed at quantifying FE or %FE need to include an N response curve with at least three data points, and treatments with organic matter inputs applied at known N rates. If the focus is on N, it is usually advisable to apply other nutrients and plant growth factors; otherwise N-FE values may reflect growth limiting factors other than N.

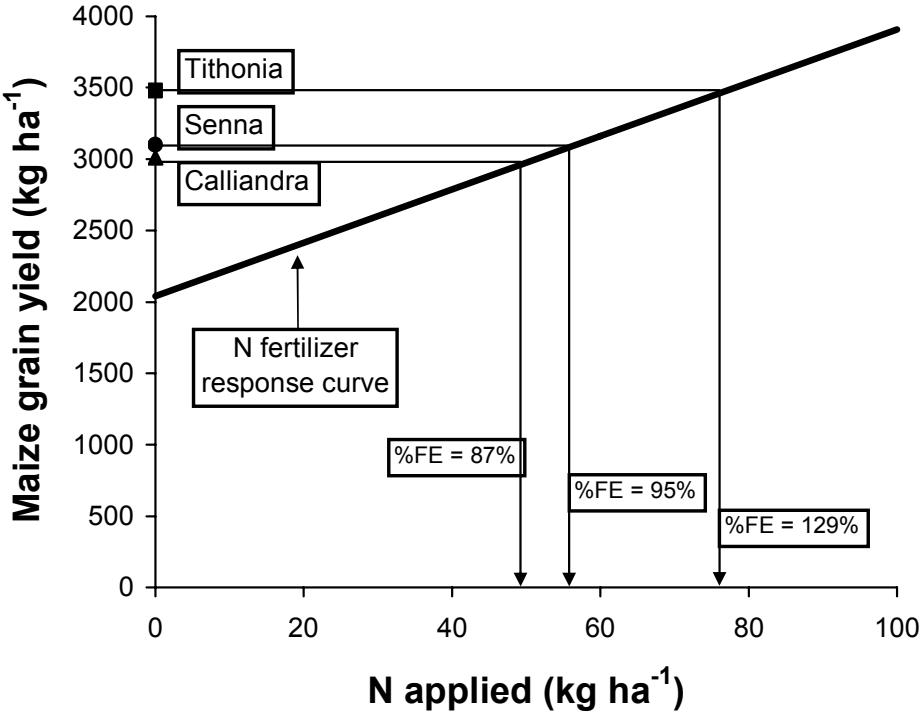

FIG. 5. Percentage fertilizer equivalency values (%FE) for three organic resources applied at Kabete, Kenya [35].

Data from field experiments in West, East and southern Africa show that the %FE values for organic materials with a low polyphenol content (<4%) and a N content >2.3% were positively related to their N content (Fig. 6). The critical level of N for increasing crop yield was 2.3%. Organic matter with a high polyphenol content (>4%) still led to positive %FE values, but the increase with increased N content was less and the N content needed to improve maize yield was 2.8% rather than 2.3% (Fig. 6). Polyphenol/N interactions seem to delay the immediate availability of N, as concluded by others from data obtained under controlled laboratory or greenhouse conditions [18, 36]. It is also apparent from this graph that the manure samples did not follow the trends shown by other organic resources, as discussed above.

Recovery of applied organic N by a first crop is usually lower than that of applied fertilizer N [37]. However, as more of the N applied as organic inputs is usually retained in specific SOM fractions, residual effects of organic resources tend to be higher than those of N fertilizer. Data from a greenhouse trial showed a significant positive relationship between the organic resource N content and the total maize-N uptake of the first crop (Fig. 7). Low quality materials such as maize stover or sawdust immobilized N, resulting in less N uptake compared to the unamended control. For the second crop, however, the relationship was negative, indicating that the medium to low quality materials provided more N to the second maize crop than the high quality materials (Fig. 7). Even in the treatment with maize stover, no further immobilization of N was observed. Only the sawdust treatment kept the N immobilized beyond the second crop. These data show that, while organic resources with a high amount of available N can immediately stimulate crop growth, residual supplies of N are larger for medium to low quality materials. More cropping cycles would be needed to judge whether cumulative yields are similar for the high and low N organic resources.

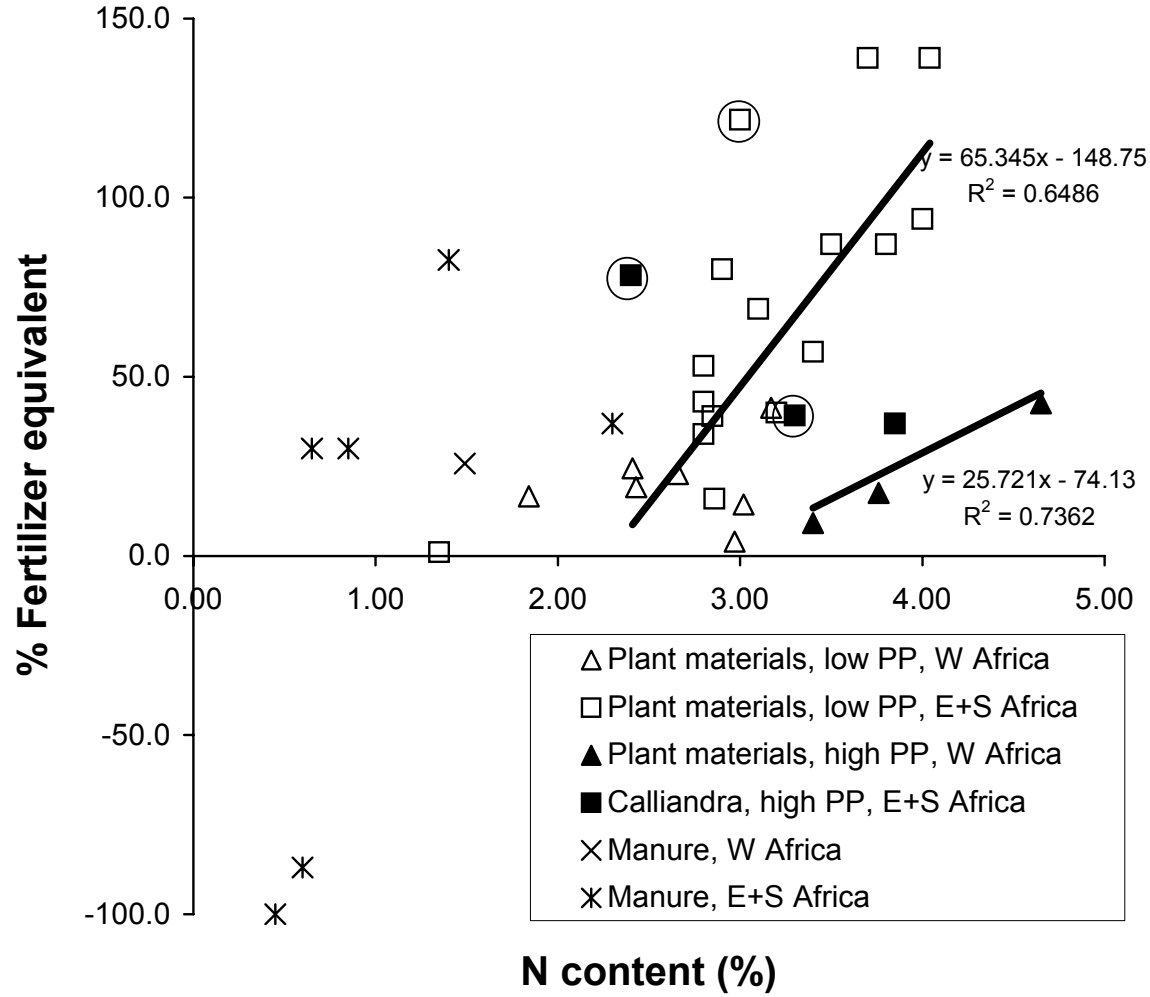

FIG. 6. Relationship between nitrogen fertilizer equivalent and the nitrogen content of plant residues and manure at a series of sites in West (W), East and southern (E+S) Africa. The linear regression equations were calculated separately for the plant materials with low and high polyphenol (PP) content; encircled values were excluded from the regression análisis [38].

As organic resources can alleviate other growth limiting factors besides N, e.g. improvement of the soil moisture status after surface application of OM as a mulch, responses to application of organic resources can often be explained in terms of such factors. These other benefits of organic resources also form the basis for the indirect hypothesis underlying potential positive interactions between organic and mineral inputs.

4.3.4.2. Impact on soil fertility

In the West African moist savanna, long term additions of OM of any origin (prunings, legume residues, manure) usually increase the soil organic C content relative to no-input controls (Table II). The soil available P, in contrast, is hardly affected by continuous application of in-situ produced OM (Table II). Although the quality of OM is usually associated with immediate decomposition and N release, evidence has been gathered that the impact of OM quality is seen also in the SOM pool. In a trial in southwestern Nigeria with ^{15}N labelled residues, Vanlauwe et al. [33] reported differences in relative residence times of

N derived from added organic matter with contrasting quality in terms of particle size SOM separates.

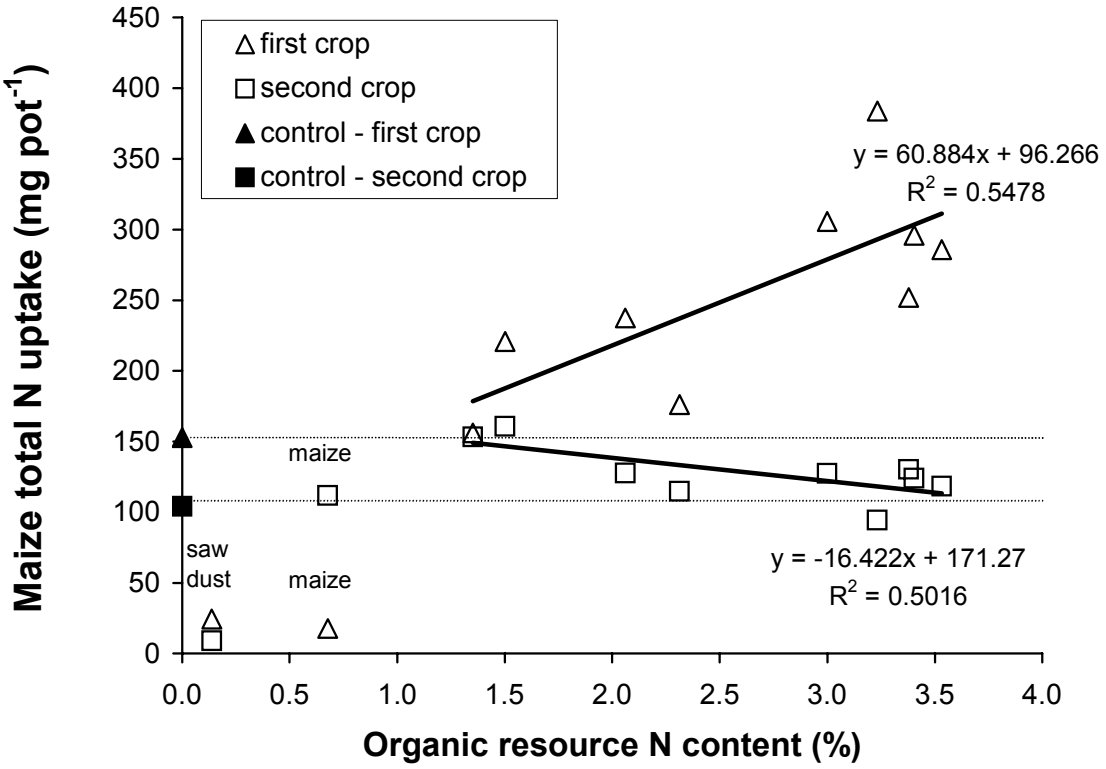

FIG. 7. Relationship between nitrogen contents of a wide range of organic resources and total (shoot + root) N uptake by maize in a greenhouse pot trial. The regression equations were calculated for all residues excluding maize and sawdust. The dashed lines give the maize total N uptake in the control soils [38].

TABLE II. CHANGES IN SOIL ORGANIC CARBON, pH AND AVAILABLE PHOSPHORUS IN LONG TERM EXPERIMENTS IN WEST AFRICA, AS AFFECTED BY APPLICATION OF ORGANIC MATTER [34]

Site (years of experiment)	Source of OM[a]	Organic C		pH[b]		Available P	
		None	OM	None	OM[a]	None	OM
		— (g/kg) —				— (mg/kg) —	
Ibadan (10)	AC[b] with *Leucaena*	5.9	9.7	5.7	5.4	10	8
	AC with *Senna*	5.9	10.0	5.7	5.9	10	11
Ibadan (10)	Rotation with *Mucuna*	5.9	7.4	5.7	5.2	10	10
Zaria (15)	External manure	NA[c]	NA	4.3	4.7	NA	NA
Zaria (45)	External manure	3.3	5.0	5.0	5.1	65	93
Saria (18)	External manure	2.5	NA	5.2	NA	NA	NA

[a] Organic matter; [b] Alley cropped; [c] Not available.

In southern Benin, Gaiser et al. [39] observed significant differences in particle size density separates of SOM after addition of organic residues of contrasting quality, and this impact was also seen in differences in N mineralization of the SOM pool. Vanlauwe et al. [40] observed higher soil-N contents in the *Senna siamea* than in the *Leucaena leucocephala* treatment in a long term alley-cropping trial in southwestern Nigeria, although OM application rates were higher in the latter treatment. In a multilocation trial in the moist savannah, the proportion of total soil-N belonging to the particulate OM pool was found to be related to the annual inputs from maize stover and prunings [41].

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CONCLUSION

The present publication has covered the topic of nitrogen management in agroecosystems. The main sources of nitrogen in crop production have been discussed, namely mineral N fertilizers, biologically fixed N and organic N sources. Within each of the three chapters on these subjects, the theoretical basis and applications of stable isotope ^{15}N tracer techniques to measure N process rates and the N balance from different N sources in various cropping systems have been elaborated.

Developing countries used more than 50 million metric tons of N fertilizer annually, worth more than US$16 billion, and most of these countries are both short of foreign exchange and depending largely on imports for their fertilizer supplies. However, due to inappropriate management practices, only a minor portion of this fertilizer is used by crops. Studies conducted using ^{15}N techniques have shown that, unless fertilizer application is managed properly, only 30–40 % of applied N is utilized by crops, and the rest is lost to the atmosphere and groundwater. Thus, it is crucial to identify management practices to minimize nitrogen losses, so that the profitability of fertilizer use can be optimized. In fact, efficient use of fertilizers is considered to be one of the major tasks in national agricultural development programmes of many developing countries. It is hoped that the present publication helps scientists in developing countries to make recommendations for farmers on the efficient use of mineral-N fertilizer and on the use of alternative N sources, such as biological nitrogen fixation and organic N resources, for crop production.

As mentioned in the Foreword, this publication was conceived as a technically oriented document for a target audience comprising soil and environmental scientists and technicians, agronomists, ecologists, extension workers, and upper level undergraduate and graduate students in these disciplines, staff of non-governmental organizations (NGOs) and other stakeholders involved in sustainable agricultural development at local, national, regional and international levels.

LIST OF CONTRIBUTORS

Hardarson, G.
Soil Science Unit,
FAO/IAEA Agriculture and Biotechnology Laboratory,
Agency's Laboratories Seibersdorf,
International Atomic Energy Agency,
Wagramerstrasse 5,
A-1400 Vienna, Austria

Hood-Nowotny, R.
Soil Science Unit,
FAO/IAEA Agriculture and Biotechnology Laboratory,
Agency's Laboratories Seibersdorf,
International Atomic Energy Agency,
Wagramerstrasse 5,
A-1400 Vienna, Austria

Jensen, E.S.
Plant Research Department,
Risø National Laboratory,
DK-4000 Roskilde, Denmark

Peoples, M.B.
CSIRO Plant Industry
GPO Box 1600, Canberra,
ACT 2601, Australia

Van Cleemput, O.
Faculty of Agricultural and Applied Biological Sciences,
Ghent University,
Coupure Links 653,
B-9000 Ghent, Belgium

van Kessel, C.
Department of Plant Sciences,
1210A Plant and Environmental Science Building,
University of California,
Davis, CA 95616, United States of America

Vanlauwe, B.
Tropical Soil Biology and Fertility Institute of CIAT
P.O. Box 30677,
Nairobi, Kenya

Zapata, F.
Joint FAO/IAEA Division of
Nuclear Techniques in Food and Agriculture,
International Atomic Energy Agency,
Wagramerstrasse 5,
A-1400 Vienna, Austria