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Use of Carbon Isotopic Tracers in Investigating Soil Carbon Sequestration and Stabilization in Agroecosystems

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USE OF CARBON ISOTOPIC TRACERS IN INVESTIGATING SOIL CARBON SEQUESTRATION AND STABILIZATION IN AGROECOSYSTEMS

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IAEA-TECDOC-1823

USE OF CARBON ISOTOPIC TRACERS IN INVESTIGATING SOIL CARBON SEQUESTRATION AND STABILIZATION IN AGROECOSYSTEMS

PREPARED BY THE
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For further information on this publication, please contact:

Soil and Water Management and Crop Nutrition Section
International Atomic Energy Agency
Vienna International Centre
PO Box 100
1400 Vienna, Austria
Email: Official.Mail@iaea.org

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FOREWORD

Soil carbon is an important component of soil that affects its physical, chemical and biological properties. Physically, soil organic carbon (SOC) improves aggregation, resulting in a better soil structure that facilitates the movement of air and water through the soil and improves root growth. Chemically, SOC facilitates nutrient availability. Biologically, SOC is the source of carbon energy for many of the soil microorganisms that drive microbial transformations in soils and is essential to enhancing soil biodiversity. Soil organic matter (SOM) acts as a major sink and source of soil carbon. Accordingly, soils and SOM currently receive considerable attention in terms of the role they can play in mitigating the effects of elevated atmospheric carbon dioxide and associated climate change impacts.

Protecting soil carbon stocks and understanding the process of soil carbon sequestration and carbon fluxes in soil are integral to efforts to manage the global carbon balance. This is because many of the factors affecting the flow of carbon into and out of soils are directly affected by land management practices. Thus, appropriate management of SOC and SOM using a combination of soil conservation and best soil management practices provides a means of enhancing the capture and long term storage of atmospheric carbon dioxide, known as carbon sequestration, and thus sustaining or increasing SOM and crop production levels, and improving environmental quality.

This publication provides an overview of conventional and isotopic methods available for measuring and modelling soil carbon dynamics. It includes information on the use of carbon isotopes in soil and plant research, including both theoretical and practical aspects of nuclear and radioisotope tracer techniques for in situ glasshouse and field labelling techniques to assess SOM turnover and sequestration, and provides up-to-date information on topics related to soil carbon sequestration and stabilization in agroecosystems. With its focus on practical application of radiotracer and stable isotope tracer techniques, it will be particularly useful for university and national research scientists working to improve soil organic matter management and conservation in agricultural systems.

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SUMMARY

The global surface temperatures have been reported to increase at an average rate of 0.06oC (0.11oF) per decade. This observed climate change known as the greenhouse effect is attributed to the emission of greenhouse gases (GHGs), including carbon dioxide (CO2), methane (CH4) and nitrous oxide (N2O) to the atmosphere, resulting in helping to trap the heat near the earth's surface causing global warming. World soils are the largest reservoir of terrestrial carbon including soil organic carbon (SOC) and inorganic carbon and that soils are a source or sink of GHGs depending on land use management.

Recognizing the urgent need to address the soil organic matter constraints for a sustainable intensification of agricultural production in developing regions of the world to ensure food security of the ever growing population, the Soil and Water Management and Crop Nutrition Section of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture decided to produce this publication "Use of carbon isotopic tracers in investigating soil carbon sequestration and stabilization in agroecosystems" as a training and reference document for environmentalists, agronomists, crop and soil scientists, and other end-users in developing Member States.

This publication comprises four papers. The first paper is a short review of soil carbon sequestration and its importance in agricultural systems and the environment, and as a potential for mitigation of climate change. This paper discusses the management of organic carbon, the capturing and storing of C in plant biomass and soils (carbon farming) in the agricultural and forestry sectors as potential strategy in enhancing soil productivity. Lastly, the initiative under the French Government, to help increase soil organic carbon pool by 0.4% per year, commonly referred to as '4 per mille' is discussed.

The second paper discusses the process and mechanisms controlling and enhancing soil carbon sequestration in fields and wetlands. Some important mitigation options for carbon sequestration are also discussed.

The third paper describes the different measurement methods for assessment of soil carbon sequestration and organic residues using different carbon isotopes. Detailed experimental procedures, measurements, calculations and data interpretation related to the use of ^{13}C natural abundance techniques, ^{14}C dating, and 'Artificial' radiocarbon (the release of artificial radiocarbon to the atmosphere as a result of nuclear weapons testing) techniques are discussed.

In the fourth paper, the use of C isotope tracer techniques and procedures for the production of labelled plant materials, continuous pulse labelling (^{13}C, ^{14}C) and equipment used has been described. The paper also provides an insight into radiation protection and safety issues in handling carbon radioisotopes. The measurement of the activities of C radioisotopes using liquid scintillation counters/analysers is briefly covered.

Six case studies are presented in the Appendix. Two illustrates the use of ^{14}C to measure carbon turnover, two are on the use of ^{13}C to determine soil carbon sequestration; one is on field measurement of plant ^{13}C uptake and distribution, and the last two demonstrate the use of ^{14}C dating and thermonuclear techniques.

OVERVIEW OF SOIL CARBON SEQUESTRATION AND ITS IMPORTANCE

J.J. ADU-GYAMFI
Soil and Water Management and Crop Nutrition Section
Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture
International Atomic Energy Agency, Vienna, Austria

K.M. GOH
Soil and Physical Sciences Group, Agriculture and Life Sciences Division
Lincoln University
Canterbury, New Zealand

M-L. NGUYEN
Group One Consultancy Limited, Optimizing Nutrient Efficiency
St Heliers
Auckland 1740, New Zealand

Abstract

Soil carbon is probably the most import component in soils as it affects their physical, chemical and biological properties. Protecting soil carbon stocks and the process of soil carbon sequestration, or flux of carbon into the soil, have become integral parts in managing the global carbon balance. Thus, recognition of the vital role played by the soil carbon could mark an important, if subtle shift in the discussions about global warming, which has been the focus on curbing emission of fossil fuel. This is because many of the factors affecting the flow of carbon into and out of soils are affected directly by land management practices. Recently soil carbon sequestration, as a potential for mitigation of climate change, has received a considerable amount of research interest. Appropriate management of soil organic matter (SOM) using a combination of soil conservation and best soil management practices provides a means of enhancing the capture and long-term storage of atmospheric carbon dioxide (carbon sequestration), and thus sustaining and increasing SOM levels and crop production and improving environmental quality. This paper presents a short overview on carbon sequestration as a potential for mitigation of climate change. The initiative under the French Government, to help increase soil organic carbon pool by 0.4% per year, commonly referred to as '4 per mille' is discussed. Details on the processes and management practices are presented in the next paper.

1. INTRODUCTION

The global surface temperatures have been reported to increase at an average rate of $0.06^{o}C$ ($0.11^{o}F$) per decade [1]. This observed climate change known as the greenhouse effect is attributed to the emission of greenhouse gases (GHGs), including carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) to the atmosphere, causing global warming.

World soils are the largest reservoir of terrestrial carbon including soil organic carbon (SOC) and inorganic carbon and that soils are a source or sink of GHGs depending on land use management [2]. It is reported that the largest active carbon pool is in soils, containing an estimated 1700 to 2500 Pg (1 Pg = 1 Gt $=10^{15}$g) of carbon, compared to 620 Pg of carbon in vegetation and 780 Pg of carbon in the atmosphere [3–4]. A review concluded that (i) long term experiments to understand feedbacks and processes of soil carbon stabilization, assess

soil carbon dynamics under different climates and land use/management scenarios, and identify policy interventions to promote the adoption of proven technologies are needed and (ii) attention should be paid to the protection and restoration of peatlands, and preservation of soils [2]. One of the strategies to manage soil organic carbon efficiently is to adopt those land use and management and practices which create positive soil ecosystem C budget [5].

Global CO_2 concentrations between October 2014 and October 2015 stand between 396.2 and 398.7 ppm (see Fig. 1 and also [6]). Concentrations of GHGs have increased by about 30% since the last two centuries [7]. Land use changes such as biomass burning, tropical deforestation and conversion of natural to agricultural ecosystems contributed about 10 to 30% of the total GHG emission since 1980 [2, 8].

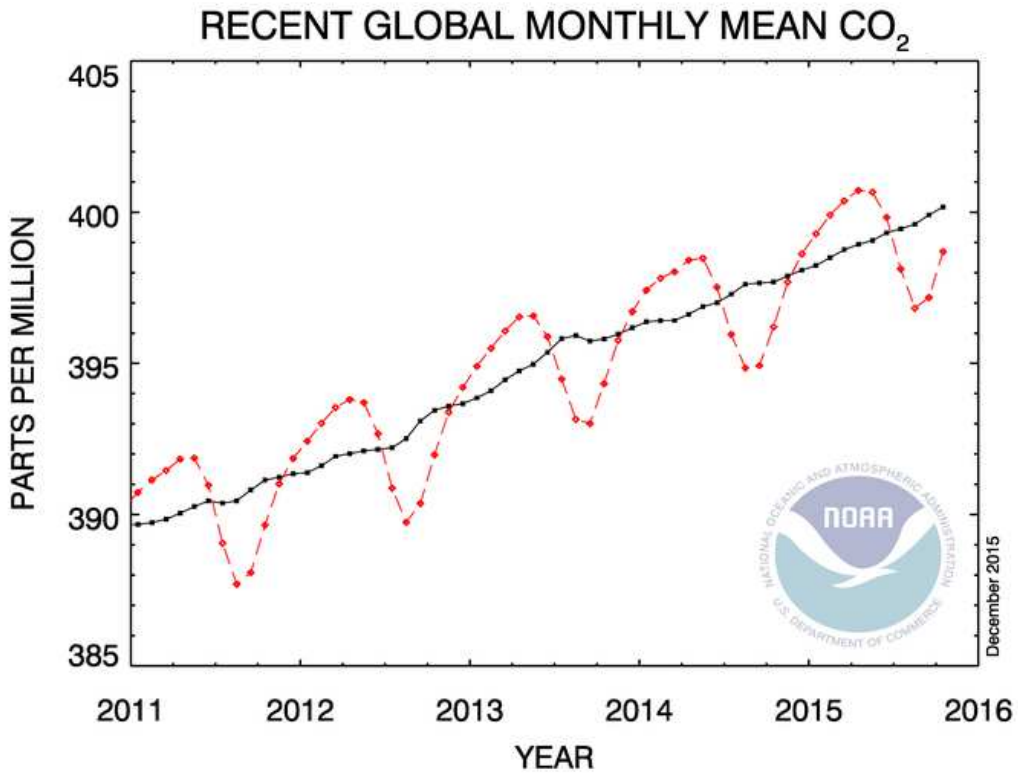

FIG. 1. Monthly mean carbon dioxide globally averaged over marine surface sites *[6]*.

2. SOIL CARBON SEQUESTRATION

The term "carbon sequestration" is used to describe both natural and deliberate processes by which CO_2 is either removed from the atmosphere or diverted from emission sources and stored in the ocean, terrestrial environments (vegetation, soils, and sediments), and geologic formations, It has been proposed as a way to slow the atmospheric and marine accumulation of greenhouse gases, which are released by burning fossil fuels [2, 9]. Data on the soil inorganic C pool (SIC) are highly variable and are estimated to be about 750–950 Pg [10]. Latest estimate from updates the global SOC mass within the top 1 m to 1325 Pg, including 421 Pg in tropical soils whereof 40 Pg occurs in tropical wetlands [11]. Global SOC

amount is just under 3000 Pg when estimates for deeper soil layers are included. Thus, a small change per unit area in the soil C pool can have important implications on the global C balance causing climate change. An increase in the soil C pool by 1 Pg will reduce the rate of CO_2 enrichment by 0.47 ppm [10].

2.1 The 4 per 1000 (quatre/mille)

This is a recent initiative under the leadership of the French Government to help increase soil organic carbon pool by 0.4% per year in the top 30 cm of soil. This proposal has been adopted globally under the auspices of UNFCC (COP–21) meeting in Paris in November– December 2015. This increase in SOC pool by 0.4% per year amounts to global SOC sequestration of (704 Pg x 0.4/100) 2.8 Pg C per year. Thus with a conversion rate of 0.47 ppm of CO_2 for 1 Pg C sequestered, the adoption of this initiative would cause atmospheric drawdown of CO_2 by 1.3 ppm per year [2, 5]. However, during a meeting of the International Year of Soils at the IAEA in Vienna on 7 December 2015, participants expressed the view that since each soil has its own properties and function, there is a need to hold regional and country meetings to explain the concept of 4 per 1000, by (i) raising awareness, (ii) doing more targeted research, (iii) promoting investments and (iv) building capacity.

2.2 Management of organic carbon

Direct sequestration of C in plants occurs when plants photosynthesize atmospheric CO_2 into plant biomass. Some of this plant biomass C is indirectly sequestered subsequently as SOM during the decomposition process. The net amount of C sequestered of a site depends on the balance between C uptake and C release. Through C sequestration, atmospheric CO_2 is reduced and SOM levels are increased.

Capturing and storing C in plant biomass and soils known as ‘carbon farming’ in the agricultural and forestry sectors is widely recognised not only as a potential mitigation strategy but also in enhancing soil productivity. The multiple benefits of SOM in food and fibre production are well documented [12–13]. Main benefits of SOM include acting as a source of major plant nutrients, a promoter of soil physical and chemical conditions, nutrient cycling processes and soil biota population. As shown in the long-term field experiments in Rothamsted, best yields of wheat and barley were obtained from plots receiving farmyard manure and nitrogen (N) fertilizer applications (Table 1 [14]).

TABLE 1. HIGHEST ANNUAL YIELDS (T HA^{-1}) OF WINTER WHEAT AND SPRING BARLEY IN PLOTS RECEIVING FERTILIZERS AND FARMYARD MANURE AT ROTHAMSTED, UNITED KINGDOM[†].

Experiment and period	Crop	Treatment		
		NPK	FYM	FYM + N
Broadbalk	Winter wheat grown continuously in rotation			
		6.69	6.17	7.92
		8.61	7.89	9.36
Hoosefield 1988–91	Spring barley grown continuously			
		5.21	5.50	6.06

[†]From [14]

Soil organic matter plays a special role in the humid tropics because soils are highly weathered and strongly leached with low cation exchange capacity and low nutrient reserves [15]. Organic matter accounts for more than 80% of the cation exchange capacity and crop productivity relies mainly on the recycling of nutrients from SOM [16–17].

Thus the increase in soil C sequestration in agricultural and forest soils results in both the mitigation of climate change and enhanced soil productivity. This has been known as providing 'co-benefits' or a 'win-win' strategy [18], reversing the damage caused by agricultural land use changes and deforestation. Soils have the capacity to sequester substantial amounts of C from the atmosphere by photosynthesis [19–20], especially in degraded soils in the United States of America [20] and in the tropics [21].

Estimates of the potential for additional soil C sequestration in soils vary widely. Enhancing SOC pool in the tropics is especially difficult in soils of the tropics with perpetual high temperatures and low input of biomass carbon because of numerous competing uses of crop residue (e.g. fodder, fuel, fencing material, construction material). It is estimated that the rate of SOC sequestration with adoptions of recommended management practices (RMP) ranges from 100 kg C/ha/year in the arid tropics to 1000 kg C/ha/yr in cool temperate climates [9]. Appropriate and strategic management of SOM using a combination of soil conservation and best soil management practices provides a means of enhancing soil C sequestration and thus sustaining and increasing SOM levels and crop production and improving environmental quality [18, 22–23]. According to FAO better farming practices could help agriculture to bury about 10% of the atmospheric C from emissions caused by human activity over the next 25 years [18].

In order to apply the Kyoto Protocol, it is essential to determine the extent and the magnitude of soil C sequestration in different regions of the world and also to be able to verify the precision of these measurements. The use of different isotopic tracer techniques to assess the decomposition rates of organic residues in soils and soil C sequestration are also presented in the other papers.

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PROCESSES, FACTORS AND STRATEGIES FOR CONTROLLING SOIL CARBON SEQUESTRATION

K.M. GOH
Soil and Physical Sciences Group, Agriculture and Life Sciences Division,
Lincoln University,
Canterbury, New Zealand

J.J. ADU-GYAMFI
Soil and Water Management and Crop Nutrition Section
Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture
IAEA, Vienna, Austria

M. L. NGUYEN
Group One Consultancy Limited, Optimizing Nutrient Efficiency
St Heliers,
Auckland 1740
New Zealand

Abstract

Protecting soil carbon stocks and the process of soil carbon sequestration, or flux of carbon into the soil, have become integral parts in managing the global carbon balance. This has been because many of the factors affecting the flow of carbon into and out of the soil are affected directly by land management practices. This paper presents an overview of the processes and mechanisms controlling, and enhancing soil carbon sequestration in fields and wetlands. Some of the important mitigation options for soil carbon sequestration are discussed.

1. PROCESSES AND MECHANISMS CONTROLLING SOIL CARBON SEQUESTRATION

1.1 Processes in soils

The balance between C additions of photosynthetic plant products to the soil and their losses determines the amount of C sequestered. Thus, processes which affect C accumulation and depletion (decomposition and mineralization or heterotropic respiration) determine soil C sequestration. Plant residues, leaves and root litter, manure, sewage sludge and other organic by-products are the main sources of C inputs in terrestrial ecosystems. The decomposition of these organic residues is a microbial-mediated progressive breakdown of organic materials with the ultimate release of end products CO_2 and nutrients to the biological circulation in the ecosystem at both the local and global scale [1–3]. Most of the C input entering the soil is labile and is emitted to the atmosphere as CO_2 (i.e. CO_2 efflux) by soil respiration by plant roots (autotropic) or micro-organisms (heterotropic) [4–5].

Plant residue decomposition involves two simultaneous and fundamental processes: the concomitant mineralization and humification of C compounds by microorganisms and the leaching downward in the soil of soluble compounds, whose C and N are progressively mineralized and immobilized [6]. Results of ^{14}C studies have shown that about one third of the C in plant residues remains behind in the soil after the first growing season in the field,

mostly as labile C and stable components of humus and two-thirds being lost mainly as CO_2 to the atmosphere [6]. The residual C becomes increasingly resistant to decomposition with time forming stable soil C [6]. However little is known globally about those biological components that drive turnover rates of litter and organic matter, and remains a major challenge [4].

Mechanisms for the synthesis of humus in soils in the humification process have been reviewed [4–5, 7]. The principal reaction involves the condensation of polyphenols and quinines. Polyphenols can be derived from plant lignin or from microbial synthesis. These are enzymatically converted to quinones, which undergo self-condensation or combine with amino compounds to form N containing polymers. The heterogeneous nature of humus is due to the fact that the number of precursor molecules and the number of combinations are so large that the formation of two identical molecules of humus from a given suite of compounds is extremely remote.

Most current theories on SOM decomposition support the view that the decomposition process follows a series of first order processes. For a given soil type and climate, the C stocks of SOM fractions (C) are directly proportional to the rate of inputs (I) expressed as:

$$\frac{dC}{dt} = d(C_1 + C_2 + \ldots\ldots\ldots\ldots\ldots + C_n)/dt = I - k_1C_1 - k_2C_2 \ldots\ldots - k_nC_n \quad (1)$$

where k = decomposition rate,

C_1, C_2 ……C_n are different fractions of the SOM,

and k_1, k_2 …….k_n are their respective decomposition rates.

This theory is incorporated in many SOM models such as the CENTURY [8], modified Roth-C [9], APSIM and SOCRATES models [10].

In addition to the humification process favouring the storage of C in soils, other processes also contribute to soil C sequestration. These include the deep rooting of trees and other plants, forming roots deep into the soil, the clustering of soil particles in soil aggregation and the movement of C within the soils by soil macro- and micro-organisms [11].

Many processes contribute to the depletion of SOC. These include soil erosion (water or wind), biomass burning and tillage (cultivation) [2, 11]. Soil erosion removes the highly enriched surface layer of soil in agricultural and grassland ecosystems or surface plant litter in forests after forest clearing, thus causing one of the most widespread form of soil degradation The erosion process is generally regarded as a four-step process involving (1) detachment and the breakdown of the soil aggregates at the soil surface, (2) transport of the detached soil particles and sediments by runoff or wind, (3) redistribution of the eroded material over the landscape, and (4) deposition of the sediments in depressional sites and protected area [5]. Other concomitant processes of soil erosion include the exposure of C locked within soil aggregates, enhancing the C mineralization by soil micro-organisms thus releasing CO_2 to the atmosphere. In addition, soil erosion decreases the ability of the soil to support plant growth, lowers soil water-holding capacity, increases runoff and soil bulk density resulting in reduced soil tilth and in some instances buries or floods crops. However, a part of the eroded soil C could be buried and sequestered [5, 12].

Biomass burning involves the use of fire as a management tool especially in forest clearing or savannas in the tropics or the clearing of crop residues before the establishment of next crop to facilitate seed germination and establishment [13]. During burning, elements

such as C, N and S are volatilized and lost to the atmosphere. The process emits numerous GHGs such as CO_2, nitrous oxide (N_2O), nitric oxide (NO) and sulphur dioxide (SO_2) [14]. Ash is produced, containing soluble nutrients and the mineralization of SOM is generally enhanced post-fire leading to further release of CO_2 and nutrients [15]. Incomplete combustion during fire results in the production of charcoal as a residue which constitutes a stable form of sequestered C. As much as 35% of the SOC pool has been reported to be present in fire-prone ecosystems of Australia [11, 16]. Fire, by removing the surface vegetation and litter, may expose the soil to erosion.

Tillage practices vary widely. However, they all involve the process of soil-stirring, loosening the soil and breaking down soil aggregates using various implements to create a suitable environment for seed germination, plant root growth, weed and moisture control. This increases soil aeration, microbial activity and the exposure of SOM to microbial attack resulting in enhancing the decomposition and loss of SOM and plant residues and the release of GHGs to the atmosphere has been extensively reviewed by [5]. After cultivation, the bacterial population increased by 20 to 30 times and fungi and actinomycetes by 2 to 3 times indicating enhanced mineralization of SOM. The decline in SOM due to cultivation occurs rapidly initially followed by a slower rate of decline, reaching a quasi-equilibrium determined by the soil, climate and management practices.

2. PROCESSES IN PADDY FIELD AND WETLAND SOILS

When a soil is waterlogged or flooded, a shift in SOM decomposition from aerobic to anaerobic transformations occur. In the absence of O_2 under the anaerobic environments, the mineralization of SOM and organic wastes result in the production of methane (CH_4) and CO_2, in addition to N_2 and hydrogen sulphide (H_2S). Two major pathways in flooded paddy field and wetland soils [17] produce CH_4 as follows:

(1) Reduction of CO_2 with H_2 (derived from an organic compound)

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (2)$$

(2) Decarboxylation (transmethylation) of acetic acid, a product of organic matter fermentation

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (3)$$

This transformation requires successive actions of different microbial populations (i.e. hydrolytic, fermentative, syntropic or homoacetonic and methanogenic microflora) [18]. CH_4 production is not only affected by the direct CH_4 producers themselves, but also by other microbial populations that influence the availability of methanogenic substrates [19]. The rate of CH_4 production over time is determined by the interactions between the various micro-organisms resulting in different phases of CH_4 production as shown in Table 1.

TABLE 1. MICROBIAL PROCESSES DETERMINING THE DIFFERENT PHASES OF CH_4 PRODUCTION AFTER FLOODING OF SOIL

Phase	Processes	Duration (d)
0	General lag phase	<1
1	Fermentation produces H_2 and acetate Iron reducers and sulphate reducers are still inactive Production of CH_4 is limited by the methanogens themselves	<10
2	Iron reducers and sulphate reducers become inactive, depleting H_2 CH_4 production is suppressed due to deficiency of H_2	<30
3	Iron(III) and sulphate are depleted Iron reducers and sulphate reducers become inactive Concentration of H_2 increases again (acetate still high) Production of CH_4 is thus possible at a relatively high rate	<30
4	Fermentation is getting limited by hydrolysis of biopolymers Acetate and H_2 reach relatively low but constant values Rates of CH_4 production decrease to relative constant value (steady state)	<100

Source: [19].

An understanding of these microscopic processes is needed to help formulating process models with better confidence [19]. Methane is also produced under anaerobic conditions in manure slurries and also as a by-product of enteric fermentation by ruminant animals [20]. In planted paddy fields, only a small percentage of CH_4 produced escapes as bubbles i.e. ebullition) through the soil and the submersion water. Most of the CH_4 (90%) is emitted to the atmosphere through the intercellular air spaces (i.e. aerenchyma) of rice plants acting as air pipes [21]. Similar processes occur in aquatic plants in temperate swamps [22].

Methane is a carbonaceous substrate for soil micro-organisms. Flooded paddy soils often contain an aerobic surface film (where CH_4 is oxidized by methane oxidizers (methanotrophs) as follows:

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O \quad (4)$$

Methanotrophs are also present in well-drained soils, especially in sites (uppermost soil layers) containing both CH_4 and O_2 where CH_4 is oxidised [23]. The draining of flooded rice fields, peatland and other wetlands introduces O_2 to the ecosystems thus resulting in CH_4 oxidation and decreases in CH_4 emissions. However, this may increase CO_2, N_2O and NO emissions especially in N fertilised rice fields. The NO is a short lived gas which influences the oxidant balance in the troposphere (e.g. ozone) and as a pollutant affecting plant growth and human health [24].

Microbial processes responsible for the production of N_2O and NO in soils are nitrification (Equation 5) and denitrification (Equation 6).

$$\underset{}{NH_4^+} \xrightarrow[O_2]{\text{Ammonium Mono-oxygenase}} \underset{}{NH_2OH} \xrightarrow[H_2O]{\text{Hydroxylamine oxidoreductase}} [NHO] \xrightarrow[H_2O]{} \underset{\uparrow \mathbf{N_2O}}{NO_2^-} \rightarrow NO_3^- \quad (5)$$

$$NO_3^- \xrightarrow[ATP]{\text{Nitrate reductase}} NO_2^- \xrightarrow[ATP]{\text{Nitrite}} [NO] \xrightarrow[ATP]{\text{NO–reductase}} \mathbf{N_2O} \xrightarrow[ATP]{N_2O\text{–reductase}} NO_3^- \quad (6)$$

In these processes, N_2O may be produced by nitrifying bacteria either during ammonium (NH_4^+) oxidation to nitrite (NO_2^-) or during the dissimilatory NO_2^- reduction when O_2 supply is limited as in flooded rice fields or wetlands.

2.1 Mechanisms of soil carbon stabilization

A strong consensus currently exists among soil scientists that not all the C accumulated in soils are protected against losses by mineralization, erosion, and leaching [11]. Only a small amount of the C accumulated can be considered to be stabilized. In terms of soil C storage, the SOC can be divided broadly into the unprotected (or labile) C with relatively short half-lives (1–20 years) and protected C with much longer half-lives (20–100 or more years). Since C sequestration applies to C stabilized in the soil for at least 20–50 years, the protected C provides the key to the control and regulation of soil C sequestration.

An understanding of C stabilization mechanisms in soils is therefore necessary in order to control and regulate SOC pools and to enhance soil C sequestration. Other than unfavourable environmental factors (e.g. unfavourable soil pH, temperature, desiccation, anaerobiosis, absence of decomposers or presence of toxic chemicals; [4–5], which retard SOM and plant residue decomposition in soils, a number of different mechanisms has been proposed by various workers as being responsible for the stabilization of C in soils. Some of these have been studied and are better known while others are yet to be studied or are at an experimental stage at present. Most studies examined only one or two mechanisms at a time. The relative importance of each of the several proposed mechanisms in a given soil and climate has not been examined. Current proposed mechanisms can be grouped into physical, chemical and biological protection mechanisms or their combinations.

2.1.1 Physical stabilization mechanisms

These mechanisms are better known than other mechanisms. They largely arose from the interactions of SOC with the soil mineral matrix, forming strong chemical bonds or causing inaccessibility of the soil C to decomposer organisms or their enzymes. The mechanisms are well documented in several early reviews [2–3, 5]. Two major groups of mechanisms have been proposed: (i) physico-chemical stabilization of SOC by sorption of organic matter to clay surfaces forming complexes and (ii) physical stabilization of SOC by the penetration of organic matter into interlayer spaces of expanding clay minerals [4]. The physico-chemical stabilization of SOC to silt and clay particles is also well documented [4, 25]. These interactions are affected by the type of clay mineral present, its specific surface area and the type of organic C present [26].

Another proposed form of physical protection of SOC by encapsulation during aggregate formation, has received greater attention [27]. This mechanism has been incorporated in many soil C turnover models as a function of soil texture [26].

Micro aggregates (>250 μm) in no tillage (NT) soils provided higher protection of SOC which may otherwise be mineralised in conventional-tillage (CT) soils (Table 2.). Stable macro aggregates in cultivated soils have been shown to contain more C and relatively younger C than the C in micro aggregates [28]. Various studies showed that tillage caused a loss of SOC due to detrimental direct and indirect effects, attributed to the mechanical (ploughing) and chemical (wetting-drying cycles) disruptions of soil structure and increased water availability and soil aeration [29].

TABLE 2. TOTAL AGGREGATE C (% WHOLE SOIL), UNPROTECTED AND PROTECTED C (% TOTAL AGGREGATE C) IN WATER-STABLE AGGREGATES IN CONVENTIONAL-TILLAGE (CT) AND NO TILLAGE (NT) SOILS AT 0–5 MM FROM THE HORSESHOE BEND EXPERIMENTAL AREA[a].

Aggregate size	Total aggregate C			Unprotected C			Protected C		
class (μm)	NT	CT	p <0.05	NT	CT	p <0.05	NT	CT	p <0.05
>2,000	895	376	*	2.77	3.51	*	0.85	0.19	*
250–2,000	329	244	*	3.11	4.52	*	0.90	0.51	*
53–106	47	37	*	1.52	2.77	*	–	–	
<53	45	77	*	–	–		–	–	

*Significant differences (two tailed t-test) between tillage treatments within aggregate size classes.
[a]Data from [27, 30].

Soil aggregation is a transient property and aggregates are continually being formed and destroyed. Aggregation is not necessarily a sequential process of binding smaller particles into larger particles but could result from the formation of micro-aggregates from micro aggregates when micro aggregates fragment depending on soil type [28].
According to [28] a larger potential for SOC sequestration was found in afforested ecosystems compared to an agricultural ecosystem under maize (Table 3.). The average annual C sequestration rates varied from 13 ± 25 g C m^{-2} for the two sites studied, similar to those reported earlier by other workers [31–32].

TABLE 3. AMOUNTS (G C M^{-2}) OF TOTAL SOIL ORGANIC CARBON IN THE A HORIZON OF AGRICULTURAL, AFFORESTED AND FOREST ECOSYSTEMS AT TWO SITES (KEMPTVILLE, CANADA; WILDLIFE, USA).[a]

Site	Afforested	Forest
Wildlife	2,491 ± 458*	5,065 ± 40
Kemptville	3,996 ± 569*	3,578 ± 505

[a]Data from [28]. Significant differences between ecosystems indicated as * p <0.05.

The SOC was stabilized for a relatively longer term within micro-aggregates (53–250 μm) and silt and clay fraction (<53 μm) in forested than in agricultural ecosystems [28]. The greater aggregation in forest ecosystems was attributed to the intra-aggregate particulate organic matter (iPOM) fractions associated with micro-aggregates and micro-aggregates occluded within micro aggregates (250–2000 μm). Using radiocarbon tracer from a local industrial release and a soil density fractionation scheme, the difference in the radiocarbon content of unprotected and protected SOM fractions increased with soil depth suggesting the importance of physical and organo mineral processes involving soil C stabilization [33].

The turnover of C in different soil aggregate fractions under different types of land use using plant residues labelled with stable isotopes (^{13}C, ^{15}N) has been reported [34]. These workers found that soil aggregate formation was associated with increased soil C storage but the results did not prove physical protection of SOM by aggregate formation since the mean age of SOC pools did not provide information on the mechanisms of SOC protection or stabilization. It was proposed that the mean age of SOC may be influenced by differences in the chemical structure and recalcitrance.

2.1.2 Chemical stabilization mechanisms

These mechanisms are attributed to the production of charcoal (or black C) by fires and biologically inert or recalcitrant and refractory compounds and very slowly decomposable humic substances (HS) and organic compounds such as lipids (e.g. waxes, cutins, suberins), and chitin by plants, soil fauna, and soil microorganisms [25, 35]. These compounds are stable due to their inherent structural stability arising from rigid alkyl structures. The compounds may be present initially in the plant and animal residues added to the soil or synthesize in situ by soil micro-organisms.

The role of black C has been implicated recently as a potential source of stabilized C in soils in Australia, Europe and Brazil [16, 36–37] and in Japanese Andosols [38]. According to [35], black C is composed of stacked layers of polyaromatic units with varying level of organisation. Using scanning electron microscopy and energy dispersive X-ray spectroscopy [39], it was reported that black C in soil did not comprise a homogenous pool but showed a great variety of shape and surface properties. Partially oxidised black C chemically interacts with the soil mineral phase, presumably conferring protection against further decomposition in soil. Due to these interactions, together with its high aromaticity and the highly condensed structure, black C is generally considered as the most recalcitrant of SOC. Estimated turnover time for charcoal is 5000 to 10 000 years [40]. Charcoal C found in buried soils in New Zealand have yielded ^{14}C dates of greater than 39 000 years [41] confirming their stability in the terrestrial environment.

Early studies of SOM have attributed the stability of humic substances (HS) to their inherent molecular structural recalcitrance such as large size, non-diffusible, disorderly structure and being copolymerized thus inhibiting their rapid decomposition by micro-organisms or their enzymes in soils [40, 42]. This is attributed the biochemical recalcitrance of HS to their intra- and inter-structural bond strengths, the degree of aromaticity and the degree of regularity of occurrence of structural units in the recalcitrant biomolecules. This recalcitrance is due to the primary molecular structure such as aromatic ring structures or changes induced by decomposition processes in the soil environment (e.g. condensation reactions in the humidification processes).

However, recent theories of soil humus formation proposed that humic substances are supramolecules consisting of associations of small heterogeneous molecules held together not by covalent bonds but by weak forces such as dispersive hydrophobic interactions (van der Waals, π–π, CH–π bonding) and hydrogen bonding in contiguous hydrophilic and hydrophobic domains of apparently high molecular sizes [43]. This unstable conformation is stabilized by increasing intermolecular covalent bonds by oxidation enzymes such as phenol oxidases (e.g. laccase) produced by soil fungi and mycorrhizas [44].

It was proposed that hydrophilic components released from microbial degradation of plant tissues or formed by microbial synthesis become progressively sequestered in the hydrophobic domains of humus and protected against further degradation. The most recalcitrant humic fractions contain mainly aliphatic or alkyl (lipid structures) compounds [45]. The hydrophobic protection was most effective in silt and clay size soil particles or larger soil particles [43, 46].

Radiocarbon dating studies [47–48] showed that pre-treatment of soils before ^{14}C dating with acid hydrolysis resulted in the acid non hydrolysable C residue which yielded the oldest ^{14}C dates.

2.1.3 Biological stabilization mechanisms

Several biological protection mechanisms and processes have been proposed but the extent and relative significance of these mechanisms are still unclear. These include the classical model of aggregate formation and organization in which micro-aggregates are bound together by roots and fungal hyphae and transient (polysaccharides) agents [4–5] the role of plant root debris and plant-derived rhizodeposits [49], laccase enzyme production by white rot fungi and mycorrhizas [44], microbial community diversity in micro-habitats [50] and the formation of refractory organic compounds by microbiota in the guts of soil anthropods. SOM is stabilised by a complex of mechanisms in biotic strategies and ecological processes that constraint decomposition rates, not based on substrate quality or soil conditions but on the biology of the decomposing organisms [51].

The organic C occluded within plant phytoliths (silicified plant opals) was highly resistant to decomposition and could be important in terrestrial C sequestration [52]. These workers found that the phytolith C constituted a substantial proportion (up to 82% of the total soil C) in the well-drained soils of Papua New Guinea after 1000 years of OM decomposition.

2.1.4 Factors controlling soil carbon sequestration

Factors controlling soil C sequestration are synonymous with those which regulate the dynamics of OM in soils, thus controlling SOM levels. These factors are well documented (e.g. [4, 5] and they result in the net gains (i.e. C sequestration occurring) or losses of SOC. Apart from climatic factors (mainly temperature and soil moisture), humidification, soil aggregate formation and pedo-translocation of SOC to the subsoil are the main processes enhancing soil C sequestration while erosion, plant residue and SOM decomposition, autrophic and heterotropic respiration and leaching deplete SOC, thus decrease soil C sequestration. Anthropogenic activities associated with soil management practices in agriculture and forestry affect these processes directly and indirectly thereby contributing to the gains or losses of C from soils. These are summarized in Table 4.

TABLE 4. SOME MAJOR SOIL MANAGEMENT PRACTICES WHICH CAUSE DECLINE OR ENHANCEMENT OF ORGANIC CARBON IN SOILS.

Decline	Enhancement
Soil erosion	Residue and manure inputs
Biomass burning and removal	Pasture establishment
Conventional cultivation	Minimum or conservation tillage
Land clearing, deforestation	Afforestation, agroforestry, alley cropping
Shifting cultivation	Fertilizer applications
Soil fertility depletion	Crop rotations with a pasture phase
Drainage of wetland	Slope and terrace stabilization
	Water conservation

According to the net effect of anthropogenic activities on soil C sequestration [53–55] is defined as:

$$CU + CF = CE + CT \quad (7)$$

where CU = carbon uptake,

CF = carbon fixation,

CE = carbon emission,

CT = carbon transport.

The effects of each soil management practice on net soil C sequestration depend on its effects on U, F, E and T. Thus, soil management practices which enhance soil C sequestration are those which have C uptake and C fixation exceeding C emission and transport. The reverse is the case for soil management practices which cause a net decline of C in soils.

2.2 Factors causing the decline of soil c sequestration

Both environmental factors (e.g. temperature) and various soil management practices (Table 4) cause a decline in soil C sequestration. Among the environmental factors, the faster turnover rate of soil C in the tropics was attributed to the higher temperature and moisture present in tropical than temperate soils which lead to enhanced SOM decomposition. The faster turnover rates for tropical soils is due primarily to the slow C pool (i.e. the silt plus clay fraction; half-life 29–38 years) compared with the active C pool (i.e. sand fraction, half-life 13 years) [28].

2.2.1 Factors due to land-use change

Land conversion causes a decline of SOM levels. For example, virgin forests accumulate more SOC than nearby plantation forests [11]. When these forests are converted for agricultural uses, SOC levels generally decline rapidly as shown in results of many long term experiments from the United States, United Kingdom, Europe, Canada, and Australia [56–58]. Factors causing this rapid loss of SOC are due to reduced and disruption of plant C inputs to soil, increased soil biological activity, soil inversion due to cultivation, and loss of high quality forest C with higher lignin content and more resistant C fractions [59].

Biomass burning, often accompanying land clearing, represents the most direct loss of crop residues (or litter) and SOC. Burning is the reverse of photosynthesis; leading to the release of CO_2 and H_2O as:

$$CH_2O + O_2 \underset{\text{photosynthesis}}{\overset{\text{burning}}{\rightleftharpoons}} CO_2 + H_2O \qquad (8)$$

Land clearing and cultivation to grow agricultural crops have been shown to decrease soil carbon by as much as 30 to 70% [1, 7, 60], loss of 20–50% of SOM as a result of clearing tropical forests and conversion into farm land. It was reported that the clearing of tropical native woodland in Tanzania resulted in 56% reduction in C and 51% reduction in soil N [61]. The loss of C under shifting cultivation is generally less than that under intensive cultivation, provided the recovery period is sufficiently long (>15–20 years) [62]. Under shifting cultivation in West Africa, the estimated soil C loss in 100 years range from 20% for a soil with a 12 year fallow cycle, to 45% for a soil with a 4 year fallow [63]. In the case of slash-and-burn agriculture, large C emissions occur due to deforestation and biomass burning.

Most of the world's burned biomass is from the tropical savannas and as two-thirds of the Earth's savanna is located in Africa [64], the African continent is now recognized as the 'burn centre' of the planet. The burning in tropical savannas is estimated to destroy annually

three times as much dry matter as that in the burning of tropical forests. The vast majority of this burning is initiated by human than lightning.

In addition to the loss of C, the immediate effect of biomass burning is the production and release into the atmosphere of many radiative and chemically active gases such as CO_2, CH_4, N_2O, NO, carbon monoxide, tropospheric ozone, methyl bromide and elemental C particulates causing human respiratory problems and ash fall over urban areas [7, 65]. On a molecular basis, bromine is about 40 times more efficient than chlorine in the chemical destruction of ozone in the stratosphere. Although the CO_2 released during burning of a forest may be sequestered by the new vegetative regrowth in the forest, the other gaseous emissions, however, remain in the atmosphere.

Ample data exist on the rates of decline of SOC and N after converting forests to agricultural use in tropical South and Central America in recent decades (e.g. [66–67]. However, no associated experimental data are available on N_2O emissions liberated from the mineralization of SOM and plant remains following forest clearing [68]. Soils in tropical forests are thought to be the largest single natural source of N_2O to the atmosphere [69].

The impact of clearing tropical forests causing the enhanced production and emissions of N_2O and NO gases is due to the forest removal increasing soil moisture and soil N availability resulting in high rates of N_2O and NO production [70]. In temperate forests large N_2O emission from an old beech forest due to N saturation by high rates of N deposition from the atmosphere over at least two decades has been reported [71]. Forests are also generally regarded as important sinks for CH_4. The conversion of forests to agricultural use or the disturbance of natural grasslands decreased CH_4 uptake [72–73].

Variable results have been reported in soil C changes in converting forests to pastures. For example, it was concluded that converting forest to pasture leads to increase in soil C stocks of about 8% on average [74]. However, the conversion could lead to large C losses (up to 50%) or net sequestration (up to 160%), [59]. These workers concluded that the variability in SOC may be due to management. Decreases in soil C are likely if C inputs to the soil are small while net C increases occur where C inputs to the soil in the newly created pasture are not impacted by herbage removal by grazing animals or low soil fertility.

Biomass burning is also practised in traditional sugar cane production, where the cane is burnt in the field a few days before harvesting to remove leaves and insects and to facilitate manual cutting [75]. This practice has been progressively prohibited by law in some areas of Brazil [76] but is still conducted in arable farming systems in New Zealand [77].

2.2.2 Factors due to tillage and soil erosion

The loss of SOM due to soil cultivation is well documented. For example, Results from the long term field experimental plots in Illinois, the Morrow Plots, established in 1876, showed that the continuous corn plot with no fertility treatment lost 45.6% organic matter in 55 years compared with that of the adjacent soil [78]. Improved soil aeration due to cultivation resulting in enhanced microbial activity and disruption of soil aggregates exposing protected SOM to decomposition together with mixing of fresh residues in the soil are largely responsible for the loss of SOM in addition to other factors [6, 79].

Farming methods which use mechanical tillage such as the mouldboard plough for seedbed preparation or discing for weed control enhance C loss. Results of long term trials at Rothamsted showed that the SOC declined under cultivation or fallow to a new equilibrium level ([56], Fig. 1.). Losses of up to 50% of SOC have been observed after 30–50 years of cultivation [32].

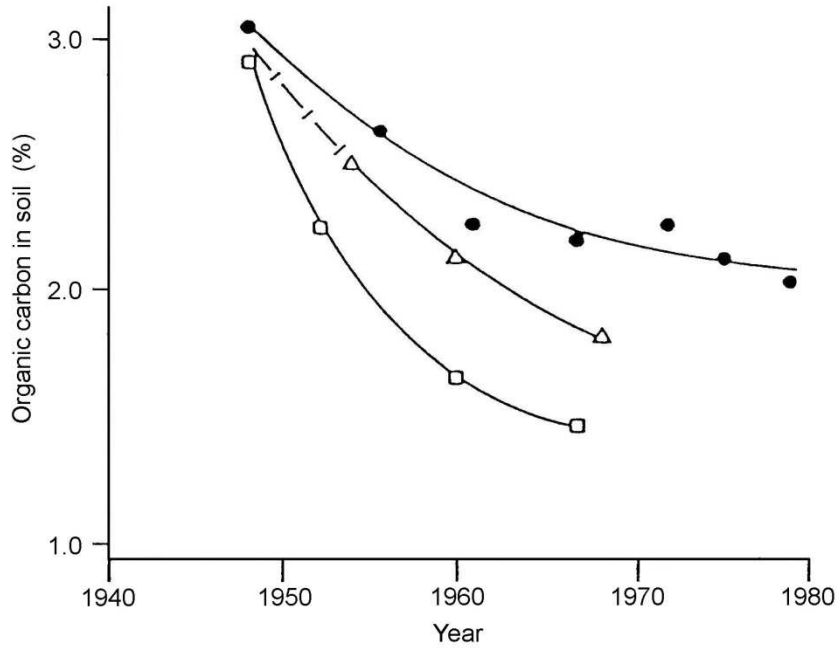

FIG. 1. Effects of different farming systems on the carbon content of Rothamsted soils ploughed out from old grass. Six-course rotation (two roots, three cereals, one year ley), •; three-course rotation (two roots, one cereal), Δ; bare fallow □. [56].

The conversion of pasture to cropland involves cultivation and this reduces soil C stocks on average by 59% [74]. Long-term agricultural use decreased C content by about 48% compared with permanent grassland [80]. The decline in SOM with cultivation is rapid initially followed by a slower rate of decline reaching a quasi-equilibrium determined by the soil, climate and land use practices (Figure 1). This is a reverse trend compared with that of SOM accumulation under natural vegetation as shown in chronosequence studies (Figure 2). Cultivation can also leave the soil more prone to soil erosion resulting in further loss of SOC [7].

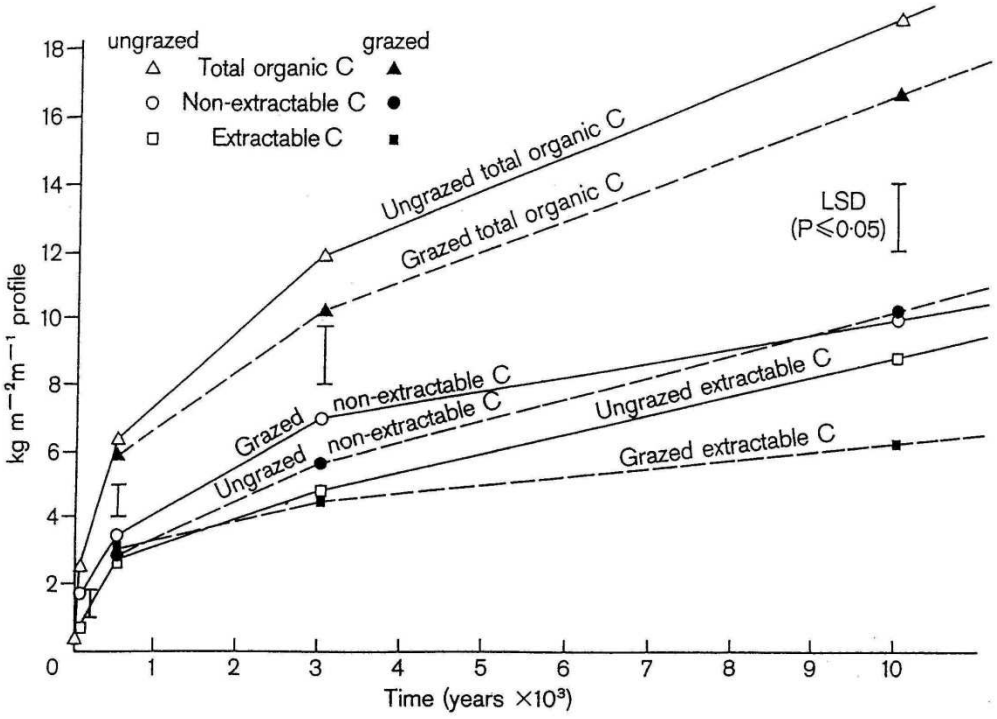

FIG. 2. Mean weights of total organic carbon, alkali pyrophosphate non-extractable and extractable organic carbon in the whole profile in a Chrono sequence of soils developed on Aeolian sand in New Zealand; ungrazed = under original vegetation; grazed = converted to pasture vegetation [80].

Soil erosion is a major cause of SOC loss and increased GHG emissions. It has been estimated that in the United States alone, water and wind erosion remove about 1.5 and 2.5 billion tonnes of soil annually [82]. The exposure of the C locked within soil aggregates after the erosion event enhances the C mineralization and the release of GHGs (e.g. CO_2 or CH_4). The transportation of the C-laden sediments to another site represents the redistribution of C over the landscape or the C loss from the eroded site but C gain at the deposition sites which are generally depressional or aquatic ecosystems. In fact, the burial of the soil C in water impoundments, lakes, bogs and other terrestrial deposition sites represents soil C storage [83]. This counteracts the loss of soil C due to C removal by soil erosion from the eroded site.

2.2.3 Factors causing the enhancement of soil carbon sequestration

Some of the major factors contributing to the enhancement of soil C sequestration due to land use are shown in Table 4.

2.2.3.1 Enhancement due to inputs of residues, fertilizers and irrigation

Increased inputs of C by adding crop residues, composts, manures, sewage sludge and other organic residues have led to increases in SOM in the soil and hence increased soil C sequestration [11, 84]. Surface-applied plant residues decompose more slowly than those that are incorporated into the soil because of less contact with the soil fauna and microorganisms [85]. About 30% of the total C input from maize residue C occurs in the 0–24 cm soil layer [86]. The quality of the plant residues and other organic materials determines their rate of

decomposition [87]. For example, crop residues with a C/N ratio below 25 are regarded as good quality residues in terms of nutrient release and are expected to result in net mineralization while residues with C/N ratio wider than 30 are termed poor quality residues resulting in net immobilization favouring soil C sequestration. Studies showed that biochemical characteristics of organic residues other than C/N ratio, such as lignin and polyphenols are also important determinants of the rate of residue decomposition [87–89]. A modified plant residue quality index (PRQIM) as:

$$PRQIM = 1/(a\ C/N + b\ lignin/N + c\ polyphenol/N) \times 100 \quad (9)$$

It was found to be significantly and highly correlated to the rate of breakdown of organic residues in terms of N release [87]. Other biotic and abiotic factors controlling the susceptibility of organic residues to decomposition include the chemical and physical nature of the soil mineral components, the ability of the soil decomposer community in using different types of organic residues and the soil environmental conditions (e.g. soil moisture, temperature).

About half of the annual global CO_2 output from soil originated from the decomposition of annual litter fall however, a vast pool of stable OM exists in soil and this decomposes very slowly over centuries or millennia [8, 90].

Increasing crop yields increase plant residue input and thus provides the potential of increasing SOC and C sequestration. Results from long-term field experiments at Rothamsted, United Kingdom showed that SOC increased with farmyard manure application over 125 years [91]. Compared with manure addition, inorganic fertilizers only increased SOM only slightly over 150 years as a result of high crop yield and crop residue input [92]. The conversion efficiencies of manure are almost twice that of plant residues due to the presence of partially decomposed products in manures [93]. These products are also present in composts from aerobic and anaerobic digestion and are hence expected to be more efficient in increasing SOC content.

The effectiveness of using composted manure as an amendment to enhance soil C storage depends on the dynamics of C mineralization and turnover. When comparing different manure management strategies, the net loss of C during manure handling and application to soil must be taken into account [94]. Improving soil fertility by long-term fertilizer (30 years) or farmyard manure (100 years) applications maintain or enlarge soil C pools [95–96], provided the ‘carbon costs’ of fertilizer production do not exceed the gain in soil C. Irrigation together with fertilizer applications have been found to be successful in increasing soil C sequestration without considering the C costs of water pumping, fertilizer manufacture and transport. The gains in C due to irrigation or fertilisation are offset by losses elsewhere in the system including the release of inorganic C from the soil due to irrigation [97].

Under irrigated pastures, an enhanced accumulation of SOC in long-term trials in New Zealand when pastures were top-dressed regularly with superphosphate fertilizer has been reported (Figure 3) [98]. An apparent steady state of SOC accumulation was obtained after about 15 to 16 years of annual superphosphate applications. A combination of cultivation and a pasture phase in an arable crop rotation system can facilitate the maintenance of SOM to a defined level of SOC as shown for soil N in Australian arable soils [99].

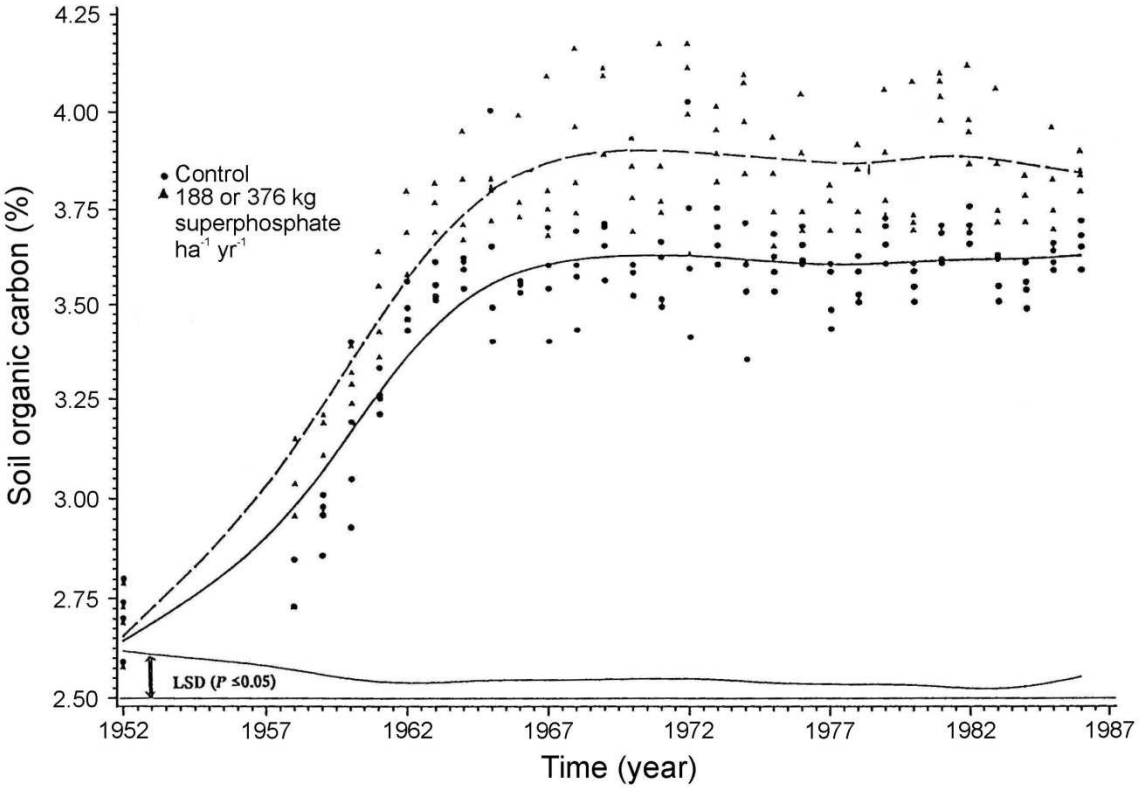

FIG. 3. Effects of long-term annual applications of superphosphate fertilizer on soil organic carbon accumulation in Winchmore, New Zealand Source: [98].

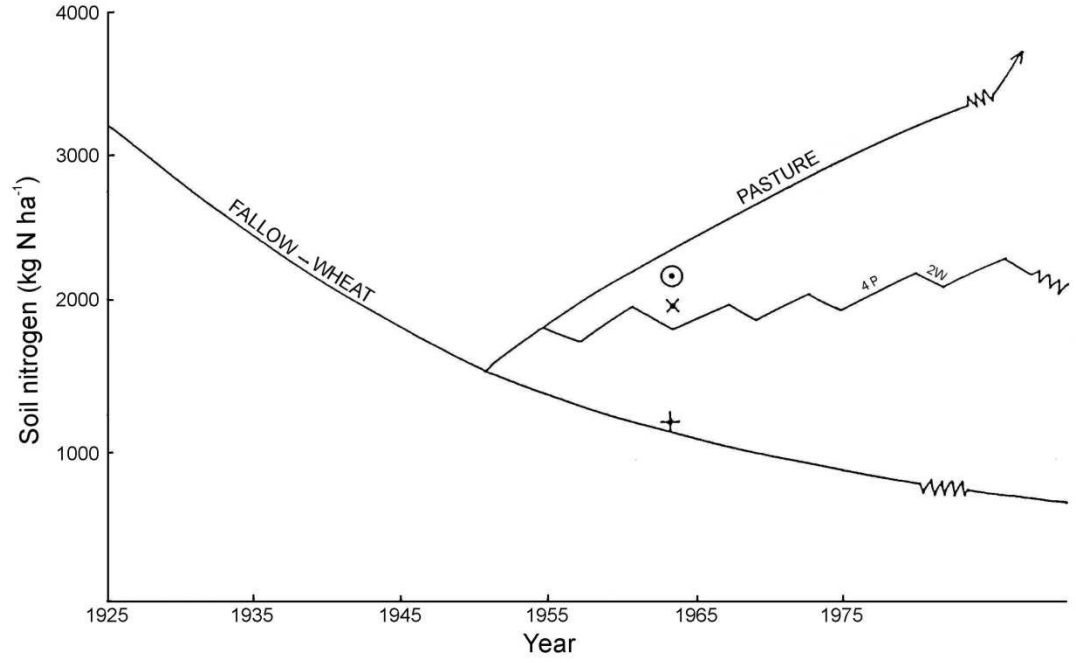

FIG. 4. Changes in soil nitrogen content under continuous fallow-wheat, and under pasture and pasture-wheat rotations in Australian arable soils. Source: [99].

2.2.3.2 Enhancement due to crop rotation and conservation tillage

Crop rotation can increase both the crop yield and the quality of biomass produced [100] resulting in high crop residues and root biomass contributing to the SOC

pool. The SOC concentration may be increased significantly by reducing the frequency of fallowing and by including a cover crop or meadow in the rotation cycle. Crop rotations which include fallow or multiple cultivations decrease soil structural stability, but growing plants with extensive root systems and with minimum tillage improves the stability of aggregates [101–104]. Aggregate stability is significantly correlated with SOC concentration due to the binding action of soil HS and other microbial by-products [105].

The use of green manures in crop rotations and cover crops showed increases in SOC as a result of increasing crop productivity. This has been demonstrated in Latin America by farmers who adopted the *Mucuna* (velvet bean) based systems of crop production. For example, in a 12-year experiment in a sandy loam, Ultisols in Benin in a maize-mucuna relay-cropping system under manual tillage with the legume cover crop *Mucuna pruriens var. utilis*, the mucuna-derived C represented more than 50% of both the litter-plus-soil and mucuna residue C based on isotopic ^{13}C natural abundance measurements [106]. However, the use of a cover crop or the substitution of green manure for grain in a 2-year rotation (6–13 years experimental duration) produced a minimum effect on soil C pools and total N but could increase the labile POM–N (i.e. particulate organic matter N) [107]. The application of either a single large (14.0 t C ha^{-1}) amendment or the annual application of compost and manure (8.1 t C ha^{-1} yr^{-1}) led to large increases in total, particulate and biomass C and N. This substantial C and N inputs from amendments are needed to significantly alter soil C and N pools in the intensively tilled, 2-year crop rotations.

Crop rotation, besides providing the benefits of lessening the build-up of pests and diseases and thus the need for C costly pesticides and herbicides, also provides an opportunity to use different crop species with varying rooting depths to aid the distribution of SOC throughout the soil profile. The use of N fixing legumes in a crop rotation [87] increases soil N and crop yield without the need of energy-intensive N fertilizers. Soils under legume-based rotations tend to preserve soil C [108]. In addition, the difference between monoculture maize and rotation was 20 t C ha^{-1} while it was only 6 t C ha^{-1} under fertilisation after 35 years. In dryland systems, legume-based rotations are generally regarded to be very valuable for maintaining soil fertility and enhancing the potential of soil C sequestration [109].

A change in conventional tillage to minimum or conservation tillage generally caused a shift in the steady state level of soil C to a higher level [110], especially in dry regions with high temperatures [62]. Minimum tillage or no till management has been shown to improve SOC, enhances soil aggregation and biological activity ([111]; see Table 5) and increased C sequestration in soils [112]. A reduction in C emissions by no-till techniques can also be attributed to low fuel consumption, less herbicide use and low machinery use.

TABLE 5. TILLAGE EFFECTS ON SELECTED SOIL QUALITY INDICATORS IN THE SOIL SURFACE (0–50 MM) FOLLOWING 12 YEARS OF CONTINUOUS NO-TILL CORN PRODUCTION [111]

Tillage treatment	Wet sieve aggregate	Total C in aggregates ($g\ kg^{-1}$)	Earthworms
No-till	45.9	24	78
Chisel	33.9	16	52
Plough	35.9	11	53
$LSD_{(0.05)}$	NS	4	18

It is difficult to quantify the effects of tillage on soil C as the effects are site (e.g. coarse-texture soils more affected than fine textured soils, and cropping system dependent [114]. For example, although rates of CO_2 loss due to tillage increased by two to fourfold, the values returned to normal after 24 hours [113–114]. Adopting a tillage approach with no ploughing did not prevent a decrease in soil C stock, although the C loss was reduced by 12% [115].

Conservation tillage using mulch tillage tends to maintain high levels of residue cover on the soil surface with less direct contact with the soil and its microbial population [115]. This reduces the CO_2 loss from the soil surface due to reduced C mineralization as found in Argentina rolling pampas [116]. In semiarid Texas in the United States, it was shown that with reduced or no tillage, the concentration of C was higher in the top 4 cm of the soil compared with that of ploughing [117]. This result is typical of organic C gains in hot climates [109], although overall rates of SOM accumulation are expected to be lower in hot climates. However, soil C increased from 15 to 32.3 tonnes ha^{-1} in four years in western Nigeria when the no tillage system was combined with mulch application [118]. Not all soils are suited to reduced tillage as some soils in the Argentina pampa lost more C under reduced tillage compared with conventional ploughing [116]. However, no tillage system provides the advantage of multi cropping thus enabling continuous or near continuous plant growth and overall higher production of plant biomass and soil C sequestration.

In the Great Plains of the United States and Canadian prairies, increased cropping frequency was found to increase soil C storage although this did not happen in unfertilized cropping systems [119]. The relationship was not linear. The gain in SOC was greater in no tilled than tilled systems due to increased plant production and C inputs and less C mineralization.

2.2.3.3 Enhancement due to grassland and its improved management

It is generally known that most rapid incorporation of C occurs when native or cultivated soils are converted to forests or improved pastures [7]. For example, the establishment of improved pastures on acid savanna soils of low fertility in tropical Colombia increased the SOM content and soil fertility of these soils [120]. Grassland, even where subject to controlled grazing, generally has higher SOC levels than cropland [121]. Furthermore, the annual C input into grassland is about twice that of cropped soils [122]. The main factor contributing to the high C storage in grassland soils is the high C input derived from the plant roots. Grasses have been shown to sequester more C than leguminous crops [123]. Although grazing is expected to remove plant residues resulting in less C sequestration, positive effects of grazing on soil C storage have been reported by various workers (e.g. [124–125]. These effects are due to grazing affecting pasture species composition and amounts of litter accumulated. For example, in the semi-arid region, higher SOC in grazed than ungrazed

soils due to increases in litter accumulation and plant species with less fibrous root system that is conducive to SOC accumulation has been reported [125].

A variety of management practices can be used in grasslands to enhance SOC accumulation. In their review, management improvements included fertilisation (39%), improved grazing management (24%), conversion from cultivation (15%) and native vegetation (15%), sowing of legumes (4%) and grasses (2%), earthworm introduction (1%) and irrigation (1%). Carbon sequestration rates were found to be highest during the first 40 years after treatments and mainly in the top 10 cm of the soil profile [126]. A report showed that short rotation grazing or management-intensive grazing was effective in enhancing rates of soil C sequestration by [127]. In temperate grasslands, practices which enhance soil C stock involve a reduction in the intensification of highly fertilised grasslands and a moderate intensification of poor grasslands [128]. Moderate N fertilizer application increases the organic C input to the soil more than C mineralization while intensive N fertilizer use induces not only a rise in production but also accelerates C mineralization and SOM decomposition [129]. Nitrogen fertilisation of grasslands decreases CH_4 consumption by the soil [130]. Plant species can also affect N_2O and CH_4 exchanges in grassland soils. For example, white clover (*Trifolium repens* L.) enhanced N_2O emissions in the grassland soil due to the N released from the degrading white clover residue in soil [131].

2.2.3.4 Enhancement due to afforestation and agroforestry

Trees are estimated to re-synthesize 10 to 20 times more C per unit area than lands under crops or pastures. However, the conversion of grassland to forest may lead to the accumulation or the release of C depending on conditions such as previous land use, climate and type of forest established [32]. Under favourable conditions, an average C accumulation of between 0.1 to 0.2 t C ha^{-1} yr^{-1} in 30 cm soil depth in the afforestation of 200-year-old grassland [132]. However, under unfavourable conditions, a loss of C has been measured after the conversion of grassland or moorland to forest [121]. In dryland, the selection of suitable tree species (e.g. *Acacia* and *Prosopis* species) can lead to viable afforestation resulting in significant contribution to soil C [133–135]. Some trees are particularly suitable on degraded lands such as *Prosopis juliflora*, which grows successfully on salt-affected soils in northwest India, showing more than fourfold increase in SOC [136].

Alternative land management practices, including agroforestry have been shown to maintain or enhance SOC storage [137], estimated that Agroforestry systems could store from 9 to 21 t Mg C ha^{-1} in semi-arid, sub humid and humid ecotones, respectively [138]. For example, it was estimated that one hectare of agroforestry in the tropics could offset 5 to 20 hectares of deforestation. This is due to agroforestry maintaining SOM levels and crop productivity and eliminating the need for a fallow period and thereby the need for further deforestation [139].

In the humid tropics, agroforestry (tree-based) systems are able to sequester C in vegetation, increasing time-average C stocks in the fields up to over 60 Mg C ha^{-1} compared to cropping/pastures, depending on the rotation age of the land use system [140]. The potential for C sequestration in the soil is less than that in the vegetation.

In the alley cropping system, leguminous trees and shrubs are integrated with food crops, and nutrients and organic matter are returned to the soil from tree pruning. This system has been shown to be a sustainable cropping system in tropical African countries [141–142]. In a Costa

Rica alley cropping system, after 19 years of agroforestry, the alley crop with *Gliricidia sepium* produced a significantly higher soil C pool compared to its respective sole crop [137] recently reported that. This is due to the greater input of organic C material from the tree pruning compared to the sole crop although C inputs in both systems were similar.

2.2.4 Factors controlling soil carbon sequestration in paddy field and wetland soils

Major factors controlling CH_4 production and emission from paddy soils have been described and reviewed by several workers (e.g. [18–19, 21, 143]. Various factors such as temperature, soil pH and addition of organic materials affect the processes of CH_4 production (Equations 2 and 3) and oxidation (Equation 4) and hence the ratio of CH_4 and CO_2 produced. The balance of these two processes determines the amount of CH_4 emitted from paddy soils and wetlands to the atmosphere. It is generally accepted that CH_4 production rates exceed actual CH_4 released from the field by factors of 2 to 4 [143].

Important factors controlling both the production and emissions of CH_4 from paddy field and wetland soils include soil properties (e.g. pH, Eh, substrate availability, physico-chemical soil properties) which affect soil microbial activities, temperature, vegetation (rice cultivars) and rice management practices controlling water content (flooding and drainage), inputs of fertilizers and other agrochemicals, manures, crop residues and other practices. Temperature, fertilisation and water status were found to be the key factors in regulating CH_4, N_2O and NO emissions from rice-wheat rotation fields in southeast China [144].

Optimum pH for CH_4 production is from 6.7 to 7.1 varying with soil types [143]. An Eh value ranging from –100 to –200 mV has been reported to be needed to initiate CH_4 production in paddy soils [145]. A negative correlation between CH_4 emission and clay content has been [146]. Higher clay content may promote soil entrapment of CH_4 [145] thus reducing net CH_4 emissions. Soil salinity affects CH_4 emission due to the presence of anions (e.g. chloride, sulphate) in saline soils. Results from incubation studies showed that sulphate-reducing bacteria could out complete methogens for substrate [147].

A strong negative correlation between annual CH_4 flux with soil salinity has been reported from coastal salt marshes in the United States [148]. Soil organic matter (i.e. organic C, organic N) and clay collectively determine the intrinsic CH_4 production in 11 Philippine soils for rice soils [149]. This is due to the inherent SOM present and the response of different soils to the added amended material and the clay protection of SOM against microbial decomposition.

The temperature dependency of CH_4 production has been reported for different soils by various workers (e.g. [146, 150]. A faster development of CH_4 production rates and higher maximum values with rising temperatures from 25 to 35°C has been reported [149]. The temperature response was roughly linear (R^2 >0.8), predicable by the Arrhenius equation as:

$$P = a e^{-E_a/RT} \qquad (10)$$

where: P = production capacity; R = gas constant; T = temperature; E_a = activation energy; a = Arrhenius constant.

Marked seasonal differences in CH_4 fluxes have been reported in most paddy soils in different agro-climate regions (e.g. [151]. In the absence of organic manures, fluxes are low in the early growth period of continuously flooded rice fields, increase gradually with some short-term peaks during mid to late season and decrease to very low levels before or after harvest.

The presence of the rice plants strongly increases CH_4 emission by providing C sources as substrates and by favouring CH_4 transfer to the atmosphere. About 30–60% of photosynthesized C by plants is allocated to the roots and a substantial proportion of this C is released or secreted by roots as root exudates [152]. Root exudates provide important C sources for CH_4 production, supplying energy to soil microbial communities including methanogens, also mobilize soil phosphorus and micro-nutrients [153].

The characteristics of rice plants have a strong impact on CH_4 emission as up to 90% of the CH_4 released from a rice field during a growing season could be emitted by rice plant-mediated transport mechanism. The presence of aerenchyma in rice plants not only allows the diffusion of CH_4 from the soil to the atmosphere but also allows the diffusion of O_2 from the atmosphere into the roots [19]. Several studies showed that most of the CH_4 (60 to 90%) emitted from paddy soils to the atmosphere is transported through the aerenchyma of rice plants rather than by the two other pathways of molecular diffusion across the water-air interfaces or the release of air bubbles. Significant differences in the methane transport capacity of rice cultivars have been observed [154]. These variations were attributed to the plant biomass, tiller number and amount/density of aerenchyma in rice plants.

Soil and crop management practices, particularly water management, fertilizer or organic matter applications and field cultural operations impact significantly on CH_4 production and emission from paddy soils. Water management is crucial for CH_4 emission because flooding the soil causes the development of an anaerobic environment due to limitation of O_2 supply from the atmosphere. When a soil is flooded, the O_2 level falls to zero in less than a day. The rate of atmospheric O_2 diffusion is 10,000 times slower through water layers or water-filled pores than through air or air-filled pores. The flooding periods in paddy soils vary in different regions and rice ecosystems (irrigated rainfed and deep-water rice). Midsummer drainage is commonly practised in Japan and China to aid the supply of O_2 to the rice plants [155–157].

In China, spatial variation of precipitation in winter (non-rice growing period) and corresponding variations of soil moisture regimes control the regional and annual variations of CH_4 emissions from paddy fields [158–159]. Methane emissions are higher in irrigated rice fields than rainfed or deep-water rice [160]. Intermittent irrigation (a few days of no water between two irrigations) and constant saturated soil moisture conditions (fields with no standing water but remain saturated) was found to reduce CH_4 emission by 25 to 58%, respectively [161]. Similar results have also been reported in India and other countries [160, 162]. Mid-season drainage, intermittent irrigation and pre-harvest field drying may reduce CH_4 fluxes if the field becomes dry. Even short-term drainage is adequate to suppress CH_4 emission as the penetration of O_2 into the soil during drainage allows the re-generation of oxidants (e.g. reduced sulphur to sulphate, ferrous iron to ferric iron) and the operation of the sulphate reducing and iron reducing bacteria [163]. These bacteria utilize acetate and H_2, the two most important methanogenic substrates more efficiently than the methanogens. The result in decreasing the H_2 and acetate concentrations and CH_4 production ceases.

Fertilizer applications affect CH_4 production and emissions by affecting the growth of the rice plant, the amount and composition of its root exudates and soil microbial activities. Nitrogen fertilizer applications reduce CH_4 emission due to stimulation of CH_4 oxidizing bacteria in the rhizosphere [164]. However, too high N fertilizer applications may lead to N_2O emission, which is a trade-off for the mitigated CH_4. Nitrate application decreases CH_4 emission due to the competitive inhibition of nitrate reduction in favour of methanogenesis [165]. This is similar to fertilisation with iron and sulphate containing fertilizers, as iron and

sulphate reduction occurs thermodynamically before CH_4 formation. Phosphate fertilisation also depresses CH_4 emission [166] as the methanogens that colonize root surfaces are sensitive to phosphate [167]. In addition, phosphate decreases the total amount of root exudation by decreasing the root/shoot ratio [166].

A large variety of agrochemicals (e.g. pesticides, herbicides, nitrification inhibitors) are known to affect microbial processes although their effects on methanogenesis and methanotropy have not been studied adequately. For example, the application of nitrapyrin (2-chloro-6 trichloromethyl pyridine) and wax-coated calcium carbide to flooded soils reduced significantly CH_4 emission [168].

The addition of organic materials (e.g. manure, green manure, compost, and rice straw and crop residues) to flooded paddy soils, in general, increases CH_4 production and emission. It lowers the Eh and supplies C to the methanogens. A single addition of rice straw can enhance CH_4 production over periods of weeks and months, only decreasing gradually with time [19].

The effects of field cultural operations (e.g. direct seeding versus transplanting; autumn versus spring ploughing; manual versus mechanical or chemical weed control) on rice growth and yield are well documented [160]. However, their effects on CH_4 emission are less established. Direct seeded rice fields are found to reduce CH_4 fluxes [161, 169]. Soil disturbance due to harrowing, transplanting, weeding, and fertilizer or pesticide application may release soil-entrapped CH_4 to the atmosphere.

Factors affecting CH_4 production and emission from wetlands are less reported than those from paddy field soils. The CH_4 emissions from typical lowland paddy fields in Indonesia, land use change from swamp and drained forest to cassava or coconut plantation lowered groundwater levels and decreased CH_4 emission, while change to lowland paddy raised groundwater levels and increased CH_4 emission [170]. However, tropical peatlands in Kalimantan, Indonesia contributed only small amounts of GHGs (i.e. N_2O, CO_2, CH_4) emissions globally to the atmosphere [171]. It has been reported that in swamps, plants with an aerenchyma favour CH_4 emission whereas plants without aerenchyma reduce CH_4 emission due to rhizospheric oxidation [18].

Organic matter is an important component of settling particles and sediments in natural lakes and man-made waterways sedimentary deposits are one of the major long-term sinks for atmospheric C and play an important role in the global C budget. However, this role has still not been quantified [173–174]. The total annual amount of C (42 mol m^{-2}) deposited in intertidal mudflat sediments in The Netherlands, 42% was buried and the remaining was emitted as methane (7%) and CO_2 (50%). Thus, better estimates of the net amount of C decline due to soil erosion are needed.

2.3 Strategies for controlling soil carbon sequestration

The Kyoto Protocol [175], which came into force on 16 February 2005 provides for C sequestration in terrestrial ecosystems. Biospheric sinks and sources of C can be included under Articles 3.3 and 3.4 of the Protocol (Marrakech Accords) in meeting targets for the reduction of GHGs emissions by comparing emissions in the commitment period (2008–2012) with 1990 baseline emissions and also allow countries to trade under the Clean Development Mechanism (UNFCCC website: www.unfccc.de). Thus soil C sequestration in ecosystems is relevant to both developed and developing countries. Article 2.3 includes land use and land management changes relating to afforestation, reforestation and deforestation while Article 3.4 relates to improved management of agricultural soils.

A vast volume of literature exists documenting the effectiveness and potentials of various land use and soil management practices for soil C sequestration in relation to soil and eco-regional characteristics [53–55, 176–180]. It is apparent from the discussions presented earlier on processes and factors affecting the losses and gains of C in soils that a wide range of agricultural, forestry and non-agricultural practices exists for sequestering organic C in soils. These practices vary considerably in their effectiveness and potentials in different eco-regions. Thus, appropriate practices differ between soil, climate and crop or plant management practices. No one single land management practice or change in isolation can mitigate the entire C needed to meet climate change commitments [179–180]. A site specific approach should be adopted in selecting the most appropriate practice to meet local needs including environmental and social implications by considering all inputs and benefits associated with implementing each input.

Integrated combinations of various soil and land use strategies are found to be more effective than a single management practice [179]. Although soil C sequestration is considered to be the most efficient and natural strategy during the first few decades of the 21st century [181], terrestrial C sequestration has a finite capacity [55]. About 80% of the global power production at present relies on fossil and nuclear fuels. Drastic reductions in C emissions are required over the next 20 to 30 years [182]. During this period, all mitigation options in reducing net C emissions are important. Terrestrial C sequestration provides a 'window of opportunity' to develop renewable sources of energy over this period [53–55].

In assessing the potentials and importance of terrestrial C sequestration [53, 178], the potential of using non-agricultural practices (e.g. buried charcoals, soil phytoliths) have not been included. These processes stored soil C for a considerably longer period than 50 to 100 years (i.e. greater than 1000 years, [11]. Further research on these aspects is needed to enhance the estimates of using soils as mitigation options.

In considering soil C sequestration options, associated side effects such as possible countervailing effects should be taken into account. A full C accounting should be considered in selecting or adopting a specific mitigation practice on the basis of net C sequestration. Climate mitigation potentials of sequestration options varied and could be significant for some management options. Gains from some mitigation potentials could be lost in practices which enhanced the emissions of other GHGs (e.g. N_2O, CH_4) [179–180]. Likewise, the drainage of peatland for agriculture and forestry reduces CH_4 emission as this is accompanied by increases in N_2O emission [14, 68]. The use of fertilizers, irrigation and manuring to enhance plant biomass production all consume energy and this should be taken into account. The gross terrestrial C sequestration must be adjusted for hidden costs [53].

Net C sequestration = gross terrestrial sequestration – hidden C costs (11)

Furthermore, N fertilizer applications may exacerbate N_2O emission and applications of manure, compost and other biosolids accentuate emissions of CH_4 and N_2O [179].

A whole farm approach for the full accounting of GHG emissions to define the success of mitigation strategies has been demonstrated [183]. A conceptual model for a livestock farm consisting of five pools: animal (1), manure (2), soil (3), crop (4) and feed (5) was developed (Figure 5) to account for the main inputs, outputs and losses of C and nutrients. The relevant direct and indirect emissions of CH_4, N_2O and CO_2 were taken into account including soil C sequestration. In addition, the potential trade off with ammonia (NH_3) volatilization and NO_3^- leaching were accounted for. This approach is an attempt for a full accounting of a

mitigation option and should be adopted in evaluating and selecting specific mitigation options for a farm or region.

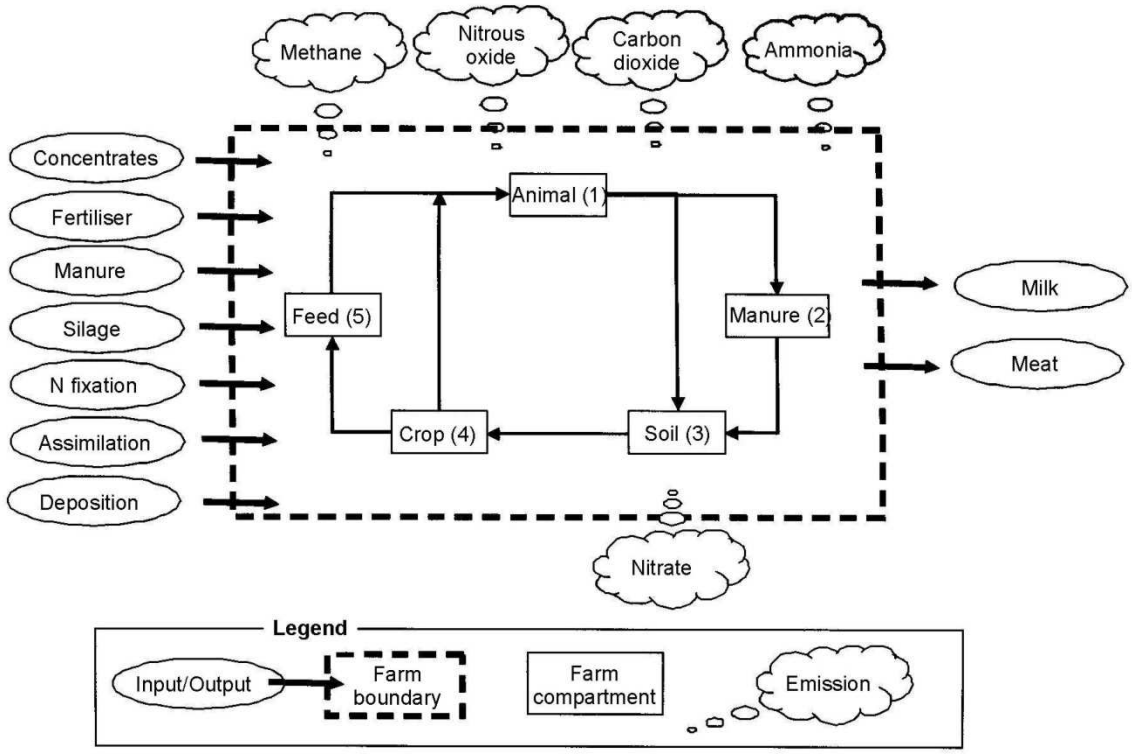

FIG. 5. Carbon and nitrogen flow for a ruminant livestock farm in The Netherlands. [183]

Although this whole farm approach provides a full accounting of GHG emissions, it did not account for energy use involving the burning of fossil fuels releasing GHG emissions of [183]. A model on the potential for SOC sequestration in England taking into account the energy savings and GHG emissions from changes in the management of tilled land and managed grassland has been reported from [184]. The changes were based on four components: (1) SOC (2) direct energy used on site (energy to power machinery and operations); indirect energy used on site (manufacture of fertilizers, chemicals, etc.); and (4) emissions of other GHG's (e.g. N_2O). The results showed that the largest C sequestration and savings were from increased proportion of woodland (afforestation). In arable management, a significant contribution to the abatement strategy occurred only if the changes were conducted in conjunction with greater use of permanent conservation field margins, increased crop residue returns and reduced tillage. In terms of true soil C sequestration, the main savings were from CO_2 and N_2O emissions resulting from reduced energy and fertilizer use, respectively.

2.3.1 Soil carbon sequestration mitigation options

Table 6 summarizes some of the important mitigation options available for soil C sequestrations and potentials based on land-use and management practices in agriculture, forestry and paddy rice fields as reported by various workers [55, 160, 177, 185]. In addition, non-agricultural and forestry practices are also included. The relative effectiveness of these management options for mitigating soil C sequestration has been discussed earlier in the factors affecting soil C sequestration. In general, the adoption of practices relating to soil

conservation and best management practices (BMPs) or management practices (RMPs) of agricultural land, particularly degraded cropland and grassland, especially in the United States [186] as well as restoration of marginal lands and wetlands/peatlands provide the greatest potential for increasing current soil C sequestration. These BMPs are those listed in Table 6. They lead to the build-up of soil C stocks by increasing the input of SOM and/or decreases the decomposition of SOM. Generally speaking, BMPs include a combination of the following: tillage methods and residue management practices; soil fertility and efficient nutrient management; erosion control; water management, and crop selection and rotation.

Conservation tillage is any tillage and planting system in which 30 per cent or more of the crop residue remains on the soil surface after planting to reduce soil erosion. The benefits of growing leguminous cover crops in maintaining and improving soil fertility and reducing soil erosion in tropical rubber and oil palm plantations, in Southeast Asia are well documented and accepted as a standard practice. Improvement management of forests in both tropical and temperature eco regions can significantly increase the plant biomass C in the standing forests and subsequently in the soil, as discussed earlier. In other situations, re-vegetation of bare, marginal agricultural land or field margins [187], biofuel production and increasing research on the development of microbial biotechnology and exploring the use of charcoals, soil phytoliths, soil carbonate deposits to store soil C is likely to lead to the establishment of improved methods for enhancing the sequestration of soil C in the long term compared with the currently predicted term of 30 to 50 years. Dedicated biomass production for biofuels by substituting for fossil fuels may play an important role over the course of the century in GHG mitigation [188] while the development of microbial technology may provide a suite of possible mitigation options ranging from direct energy production to C capture and enhanced energy efficiency across industrial sectors [189]. In paddy field soils, various practices have been suggested as effective means for reducing CH_4 production and emissions. These have been discussed earlier in the factors affecting soil C sequestration in paddy field soils. In general, a combination of methods is advocated [160]. These include water management, selection of appropriate rice cultivars and fertilizers, use of nitrification inhibitors (e.g. nitrapyrin, calcium carbide), and the use of improved methods of applying organic manures, composts, crop residues, green manures and fertilizers and also field cultural practices. Major effective strategies which have been identified as being significant and reliable for paddy field soils across regions [151, 190] are:

- Direct seeding;
- Mid-season drainage and intermittent irrigation;
- Composting organic amendments such as rice straw before incorporation and combined application of chicken manure and urea;
- Supplying N, P and K using sulphate-containing fertilizers and amendments as gypsum due to the inhibitory effects of SO_4^{2-};
- Breeding and selecting appropriate rice cultivars.

TABLE 6. SOME IMPORTANT MITIGATION OPTIONS FOR SOIL CARBON SEQUESTRATION.

Ecosystems	Practices
Forestry	Afforestation, reforestation, improved forest management, establish perennial vegetation, and appropriate harvesting practices.
Arable farming (croplands)	Conservation, minimum or zero tillage, and mulch tillage. Rotation with pastures, deep rooting crops, legumes and high residue yielding crops. Convert cropland to pastures. Growing cover (leguminous) crops. Erosion control with conservation tillage, buffer strips, riparian filters, stabilization of slopes, contour plantings, etc. Integrated fertilizer management and irrigation. Integrated pest management to reduce hidden costs. Improve efficiency of crop residue, compost and manure use.
Pastoral farming (grazing lands)	Controlled grazing. Improve efficiency of animal manure use, crop residue use and livestock management. Improved forage species, incorporating legumes and deep rooting species. Integrated fertilizer management. Using sewage sludge and farm effluents. Soil and water conservation. Zero or prescribed burning.
Dryland farming	Improved use of zero-tillage, green manures, farmyard manures, fertilizers, cover crops and rotation cycles. Use agroforestry with trees and alley cropping systems.
Paddy fields	Water management. Selection of appropriate rice cultivars. Improved methods of applying plant residues, manures and fertilizers. Improved field cultural operations.
Wetlands	Control drainage. Create and protect wetlands.
Urban forest and grassland	Create and protect these ecosystems.
Tundra and taiga	Conserve and protect the system.
Other non-agricultural land	Re-vegetate bare land with trees or appropriate plant species. Retire marginal agricultural soils and convert to shelterbelts, hedgerows, vegetate field margins, growing biofuel crops. Research the use of microbial biotechnology and also charcoal, soil phytoliths and inorganic carbonate deposits to improve longer term (i.e. greater than 50 years) soil C stabilization as potential mitigation options. Enhance biodiversity.

It is feasible to select and breed high yielding rice cultivars with a reduced CH_4 transport capacity with the advent of recent techniques for measuring CH_4 transport capacities, plant traits such as tiller numbers, biomass, and development patterns of aerenchyma formation [160]. In contrast to paddy field soils, at present there is inadequate information on managing SOM in wetlands, Histosols (e.g. peatlands, swamps, bogs, marshes, mire, fens) and other aquatic ecosystems other than protection and creation of more wetlands. A significant proportion of global C is stored as peat, with high altitude peatlands in the northern hemisphere alone estimated to contain about one-third of the global soil C pool [191]. Annual rates of C sequestration in coastal marsh soils are estimated to be between 0.05

to 0.5 kg C m^{-2} yr^{-1} [192]. Because marsh soils are continually accreting (2 to 8 mm yr^{-1}) to keep pace with apparent rising sea level, C sequestration occurs at a relatively rapid rate shown as steep slopes of C sequestrating rates (Figure 6). On a per hectare basis, the long-term potential for C storage in coastal marsh soils far exceeds that of upland soils. Drainage of organic soils for agriculture, forestry or fuel production worldwide has led to substantial amounts of C being lost to the atmosphere [193]. Drainage may also decrease CH_4 flux [194] due to lower ground water table resulting in a thicker aerobic top soil layer, thereby decreasing CH_4 production and increasing CH_4 consumption. The factor responsible for oil palm (*Elaeis guineensis*) ecosystem acting as a sink for CH_4 in contrast to sago (*Metroxylon sagu*) and tropical forest ecosystems [194]. More research on understanding C sequestration in various aquatic ecosystems as well as in lakes, groundwater and various freshwater and seawater bodies is needed [195].

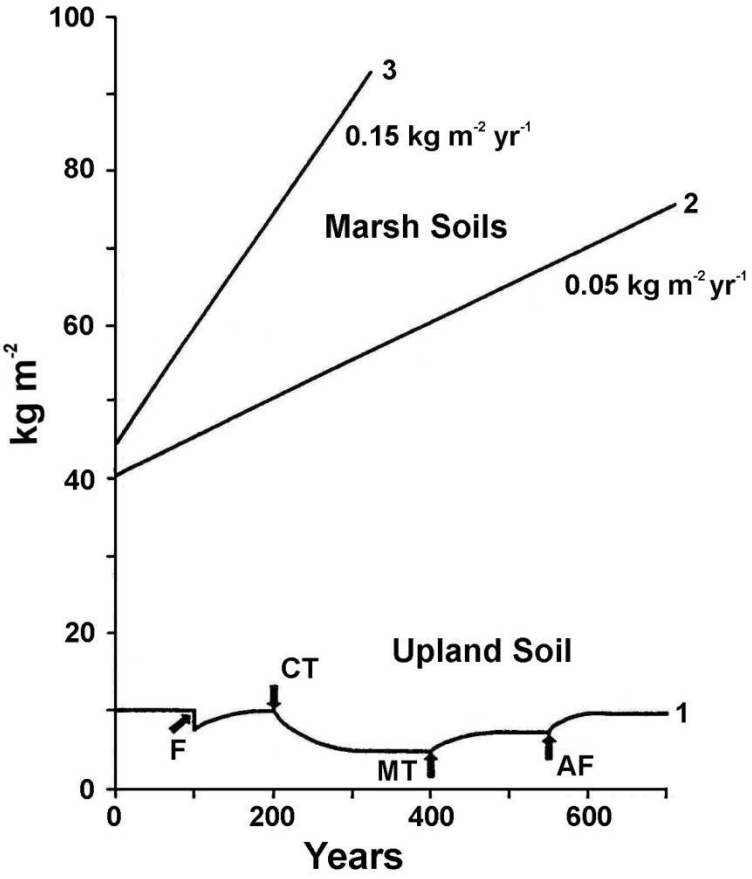

FIG. 6. Carbon storage and sequestration in an upland soil (1) under various natural and managed conditions, and in marsh soils (2 and 3) under two rates of carbon sequestration; F = fire; CT = conventional tillage; MT = minimum tillage; AF = agroforestry. [192]

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ASSESSMENTS OF SOIL CARBON SEQUESTRATION AND DECOMPOSITION OF ORGANIC RESIDUES USING DIFFERENT CARBON ISOTOPES

J. J. ADU-GYAMFI
Soil and Water Management and Crop Nutrition Section
Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture
IAEA, Vienna, Austria

K.M. GOH
Soil and Physical Sciences Group, Agriculture and Life Sciences Division
Lincoln University
Canterbury, New Zealand

Abstract

A variety of techniques involving the use of different C isotopes (e.g. ^{13}C, ^{14}C) are used to determine the decomposition or turnover of SOM. In this paper, tracer methods involving labelled (^{14}C or ^{13}C) plant materials, ^{13}C natural abundance, the ^{14}C dating and the "artificial radiocarbon" (the release of artificial radiocarbon to the atmosphere as a result of nuclear weapons testing) techniques are discussed. Different soil organic matter (SOM) simulation models are compared and the need for further validating and testing the accuracy of the conceptualized simulation SOM models with field-derived data is highlighted.

1. INTRODUCTION

Under the Marrakech Accords of the Kyoto Protocol, Articles 3.3 (activities limited to afforestation, reforestation and deforestation) and 3.4 (activities limited to forest management, cropland management, grazing land management and re-vegetation) allow the biospheric sinks and sources to be included to meet reduction targets of greenhouse gas emissions for the first commitment period of 2008–2012 of the Protocol [1]. Thus, methods for the assessment, monitoring and verification of soil C changes under these Articles are needed.

In simple terms, soil C sequestration over a two time period (e.g. commitment period of 2008–2012) may be defined as the difference between C addition and C loss [2] and for known amounts of C input as:

$$\text{Soil C sequestration} = \text{C input} - \text{C loss (or turnover)} = I - kC \qquad (1)$$

where: I = C input,

k = decomposition rate or constant.

Two approaches are generally used to measure C loss based on mass balance measurements. This is either by determining the net C retained (i.e. C stocks) at the end of the period or the turnover rate of C input at each of the year for the period. Alternatively, C losses or accumulations on land can be determined by measuring soil C stocks at the beginning of 2008 and at the end of 2012 (i.e. first commitment period) or C fluxes into and out of the soil in a given ecosystem over the 5-year period. Although flux measurements are very sensitive, the flux method is technically demanding and expensive and is predisposed to compensating errors [1, 3]. It is suggested that these two different methods be used, one for measuring C

accumulations or losses of C, while the other method as an independent method to verify the change [1].

A summary of measurement methods for assessing C accumulations on land is presented in Table 1[1, 4]. Many countries (e.g. Australia, Canada and the United States) are proposing to use a combination of direct measurement, existing inventories (e.g. soil inventories or soil maps), remote sensing and simulation models to estimate C gains or losses on land [5–9].

Equations 2 and 3 were used to calculate the size of SOC pool [10] as:

$$\text{Soil pool (kg ha}^{-1}\text{)} = \text{soil depth (m)} \times \text{bulk density (Mg / m}^3\text{)} \times 10{,}000\ (\text{m}^2\ \text{/ha}) \times 1{,}000\ (\text{kg / Mg}) \quad (2)$$

$$\text{SOC pool (kg / ha)} = (\text{g SOC /kg} / 1000\ \text{g}) \times \text{soil mass (kg / ha)} \quad (3)$$

The difference in SOC pools measured at the beginning (baseline) and the end of the period is the amount of net change in soil C or soil C sequestration [11].

TABLE 1. MEASUREMENT METHODS FOR ASSESSING ACCUMULATIONS OF CARBON ON LAND [1].

Stock change measurements methods

- Vegetation inventory
  - Stem wood volume – forest inventory
  - Total tree biomass – allometry
- Wood products – models of wood products
- Soil and litter
  - Woody debris – volume and mass measured
  - Litter-sampling and carbon analysis – highly spatially variable
  - Mineral soil-sampling and carbon analysis – highly spatially variable

Flux measurement methods

- Chambers, eddy covariance – for scales less 1 km^2
- Tall towers, balloons for convective boundary layer budgeting – landscape, regional scale
- Flask measurements and flux measurements from aircraft; coupled with inversion analysis – continental scale

Remote sensing to determine geographical extent and change

- Current resolution is 1 km^2 but 30 m possible soon
- Geographic extent possible, vegetation type possible, residue over, tillage, and perhaps soil organic carbon and moisture content of bare soil will become possible in near future

Models

- To be used in combination with the above methods

The accuracy of the mass balance method for detecting the net changes in the soil C pool as a measure of soil C sequestration depends on accurately measured soil bulk density. Soil bulk density changes with time, and land use. Several methods are used for determining soil bulk density [12]. A standardized procedure with a specified time of sampling is needed. Furthermore, quantifying soil C changes using mass balance approach is difficult as the annual changes are small compared with the background C levels already present and the spatial variability is very large [3, 13–15]. Estimations of SOC at landscape level are also important due to landscape variability [16].

The smallest detectable difference using the mass balance approach to measure net C changes was about 1 t C ha^{-1} (2–3% of background C) with adequate statistical power (90% confidence level) [17]. This requires a very large sample number (>100). With a reasonable sample number of 16, the minimum detectable difference was 5 t C ha^{-1} (10–15% with) background C, 90% confidence level) [1]. Many agriculture practices may not cause soil C accrual rates as high as this during the 5-year commitment period [18]. Locally calibrated models and stratifications are used to reduce costs and improve the reliability of the baseline and follow-up survey data. However, direct soil sampling will still be required. Thus the verifiability of the data obtained based using the mass balance approach could be difficult, expensive and prohibitive at the national level [1, 19–20].

An example of the soil sampling required for verification in a case study is shown in Table 2 and Figure 1 [21] of three different fields (i.e. cultivation, fallow, and grazing). It shows that the number of soil samples required to verify changes in soil C stock over time varies according to:

(a) The spatial variability of the soil C stocks in a site;

(b) The minimum change of C stock that must be detected;

(c) The statistical level of significance that must be obtained.

TABLE 2. MEASURED DATA (GM^{-2}) OF SOIL ORGANIC CARBON AT 0–20 CM SOIL DEPTH FOR N = 100 SAMPLES FROM EXPERIMENTAL SITES IN A SUDAN CASE STUDY SHOWING STATISTICAL VARIABILITY[21].

SOC, 0–20 cm, (g/m^2) (n = 100)	Cultivated	Fallow	Grazing
Mean ± standard deviation	519.2 ± 461.5	532.3 ± 455	411 ± 226.8
Median	374.7	426	367.9
Minimum, maximum	242.9, 3 716.3	239.5, 4 277.5	181.4, 2 303
Variance	212 952	207 043	51 425
Texture (%) sand, silt, clay	93.7, 3.6, 2.7	95.1, 3.0, 1.9	93.6, 3.2, 3.2

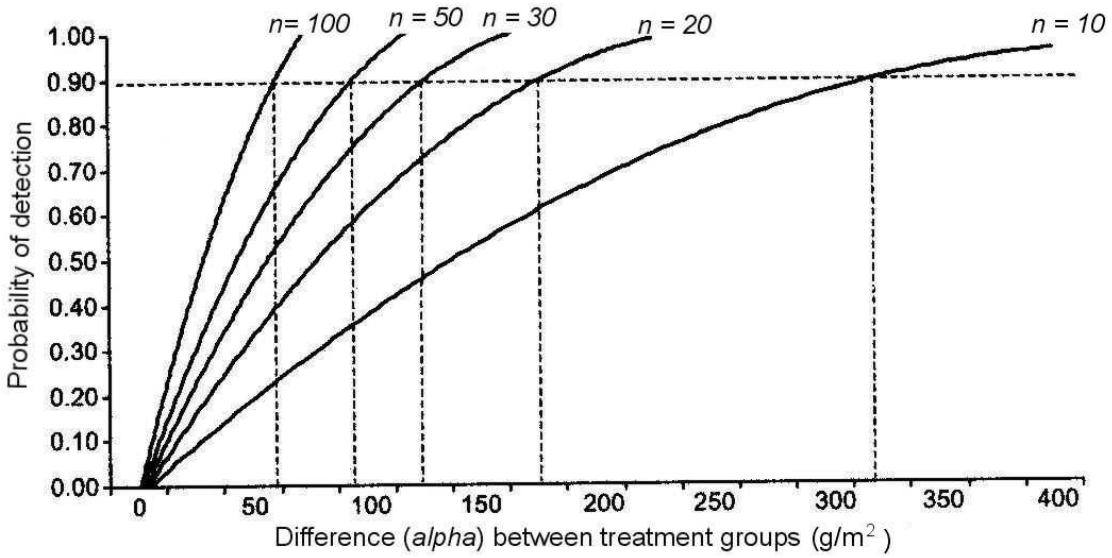

FIG.1. Probabilities of detecting differences for different sample size (n) based on the data obtained in a Sudan case study by [21].
NOTE: The dotted lines indicate the differences detectable 90 percent of the time with a Kruskal-Wallis test (testing at significance level $\alpha = 0.05$) for five sample sizes (n = 10, 20, 30, 50 and 100).

Techniques involving different isotopes (e.g. ^{13}C, ^{14}C) have been used extensively in studies of SOM dynamics such as soil C accumulation, turnover of recently added C and sizes of different SOC pools [22] but these methods have not been adopted for general use in assessing soil C sequestration by IPCC (2000) as shown in Table 2. Although isotopic techniques are also affected by soil variability similar to the mass balance methods, the main advantage of the isotopic approach is its ability to detect small changes in soil C stocks against a large background soil C. In addition, with this approach, the amount of tracer entering the system is exactly known. This provides a means of following the gain or the loss of new C entering the soil [23]. Different C isotopes (e.g. ^{13}C, ^{14}C) are used in techniques for determining SOM turnover and the decomposition of labelled organic residues in soils. The SOM is regarded as synonymous to SOC as SOM = 1.724 SOC, assuming SOM is 58% C. These measurements are used to quantify C losses, which can then be used to determine soil C sequestration according to Equation 1.

2. DETERMINATION OF SOIL ORGANIC MATTER DECOMPOSITION AND TURNOVER USING CARBON ISOTOPES

A variety of techniques involving the use of different C isotopes (e.g. ^{13}C, ^{14}C) are used to determine the decomposition or turnover of SOM-C in widely known studies of SOM dynamics (e.g. [23-28]). Few attempts have been made to incorporate the decomposition or turnover value obtained to represent C loss for calculating soil C sequestration according to Equation 12. These data are often used to develop simulation models of SOM decomposition or turnover. The turnover values obtained in these models can also be used to represent C loss for calculating soil C sequestration (Equation 1).

The turnover of an element in a pool is defined as the balance between inputs (I) and outputs (O) of the element to and from the pool [29]. A first order model (Equation 1) is generally used. Under a steady state condition [30–31] with a constant proportional C mass loss per unit time and a zero-order C input, the rate of change of soil C stock (C) is defined as:

$$\frac{\delta C}{\delta t} = I - kC \qquad (4)$$

where t is the time, k is the decomposition or turnover rate (Equation 1), and kC, the output (0) or C loss in Equation 1 (Equation 4 is a summarized form of Equation 1). The turnover is often quantified as the mean residence time (MRT) or the half-life ($T_{½}$) of the element. Assuming equilibrium, (I = 0) and MRT is calculated as:

$$MRT = 1/k \qquad (5)$$

The MRT is defined as either the average time the element resides in the pool at steady state or the average time required to completely renewing the content of the pool at steady state. The MRT is related to half-life ($T_{½}$) as:

$$MRT = T_{½}/\ln 2 \qquad (6)$$

The unknown decomposition rate, k, can be determined by one of the five different methods using the same or different C isotopes (^{13}C or ^{14}C):

(i) Tracer techniques

(ii) ^{13}C natural abundance technique

(iii) ^{14}C dating technique

(iv) Artificial radiocarbon technique

(v) Soil organic matter models.

2.1 Tracer techniques

Tracer methods for determining the SOM decomposition or turnover rate involve the production of ^{13}C or ^{14}C-labelled plant residues in growth chambers or in situ labelling of plants in the field using continuous or pulse labelling (e.g. 32–36]. These procedures for producing labelled plant materials are described elsewhere.

In tracer methods involving labelled (^{14}C or ^{13}C) plant materials (e.g. whole plants or roots), the labelled materials are added to soils in incubation experiments [27] or to soil cores in the field [37]. The decomposition of labelled plant material releases CO_2 and the labelled CO_2 are captured in alkali (e.g. NaOH) in enclosed jars or chambers and its radioactivity assayed. The fraction of the ^{14}C-labelled (or ^{13}C-labelled) CO_2 produced from the added labelled plant material (e.g. root) represents the decomposition or the loss of C and is defined for added labelled roots, as an example, as:

$$\%\ \text{root}\ ^{14}C\ \text{respired} = \left(\frac{^{14}C\ \text{respired (Bq)}}{^{14}C\ \text{added (Bq)}}\right) \times 100 \qquad (7)$$

This gives the decomposition rate when divided by the total duration time of the experimental period. It is then extrapolated to one year period for determining the annual decomposition rate, k. These data are generally used to develop simulation models, and also to calculate k value. These models will be presented later.

Alternatively, the ^{14}C-labelled plant material is added to soil samples in micro lysimeter tubes which are then inserted in the field [38–40]. The amended soils are then left in the field for a few years (e.g. 2 to 5 years) under field conditions. Soil samples are periodically destructively sampled and total ^{14}C remaining in the soil is then determined. The amount of ^{14}C remaining in the soil at the final or periodic sampling represents the amount of C retained by the soil over the total time period or periodical sampling intervals after the addition of the ^{14}C-labelled material to the soil. The loss of ^{14}C-labelled plant residue represents at each sampling time the loss due to residue decomposition at the time after the addition of the labelled plant material to the soil.

Using in situ experiments, plants are pulse-labelled with ^{14}C or ^{13}C in the field or growth chambers [34, 36, 41]. The incorporation of the ^{14}C or ^{13}C into the soil or plant roots is determined after labelling by destructive sampling of the soil or roots followed by ^{14}C or ^{13}C analysis. The plants are allowed to grow for different lengths of time (e.g. one year under field conditions) and the soil or roots are sampled periodically to determine the amount of ^{14}C or ^{13}C remaining in the soil or roots.

Using ^{14}C plant roots as an example, in order to calculate the decomposition rate k of roots, the amounts of ^{14}C remaining in roots at different sampling times are plotted against time, assuming Day 0 for the first sampling after labelling and a first-order decomposition rate. The net change of root ^{14}C at any time (V) is assumed to be proportional to the amount of residue root ^{14}C present (first order decay model) at that instant as expressed by Equation 8:

$$V = -dC/dt = kC \qquad (8)$$

where k is the decomposition rate,
C is the concentration of ^{14}C of the residual root at the instant of time t.

The solution of Equation 8 gives:

$$C = C_o e^{-kt} \qquad (9)$$

where C_o is the initial ^{14}C percentage at Day 0 in the roots (100%). The rate constant k is estimated by a non-linear least square method and the goodness of fit is tested by R^2 (the coefficient of determination). Other models can also be developed by using the data obtained and tested for goodness-of-fit. The half-life of roots can be obtained by Equation 10 as:

$$T_{½} = 0.693/k \qquad (10)$$

2.2 ^{13}C natural abundance technique

The principle of this technique is based on the discrimination of ^{13}C and ^{12}C isotopes during CO_2 assimilation by plants (i.e. photosynthesis) with different photosynthetic pathways [41-44]. The $^{13}C/^{12}C$ ratios are expressed as $\delta^{13}C$ values where:

$$\delta^{13}C\text{‰} = \frac{^{13}C/^{12}C \text{ sample} - {}^{13}C/^{12}C \text{ standard}}{^{13}C/^{12}C \text{ standard}} \times \frac{1000}{1} \qquad (11)$$

The standard is carbonate from Pee Dee belemnite limestone from the Cretaceous Peedee formation of South Carolina, U.S.A. and the units are mille (‰). Atmospheric CO_2, plant material and SOM are depleted in ^{13}C relative to the standard and therefore have negative $\delta^{13}C$ values. The more depleted in ^{13}C a material is, the more negative the $\delta^{13}C$ values will be.

The ^{13}C natural abundance technique relies on:

(i) The difference in ^{13}C natural abundance (i.e. δ^{13} values) between plants with different photosynthetic pathways (C-3 plants with Calvin cycle vs C-4 plants with Hatch-Slack cycle)

(ii) The assumption that the ^{13}C natural abundance signature of SOM is identical to the ^{13}C natural abundance signature of the plants from which it is derived [22–23, 42, 44].

In C-3 plants, the enzyme rubisco during photosynthesis leads to the ^{13}C depletion of about –27‰ (–35‰ ≤ $\delta^{13}C$ ≤ –20‰) when compared with atmospheric CO_2 ([45]. In C-4 plants, the enzyme phosphoenol pyruvate carboxylase during photosynthesis results in ^{13}C depletion of about –13‰ (–15‰ ≤ $\delta^{13}C$ ≤ –7‰). The $\delta^{13}C$ values of different plants have been reviewed by [46–47]. The assumption is that when plant residues are converted to SOM, the effects of humification and other microbial-related processes on $\delta^{13}C$ values are negligible.

Assuming that $\delta^{13}C$ values in SOM remains close to the original vegetation, soils developed under C-3 plants (e.g. wheat, cool season grasses, forest vegetation) contain SOM with $\delta^{13}C$ = –27‰ whereas soils under maize and C-4 grasses contain $\delta^{13}C$ = –12‰ [43, 48]. The method is based on growing C-3 plants on a C-4 soil or vice versa. Under this condition, due to a vegetation change at some known point in time, the rate of loss of the C derived from the original vegetation and the incorporation of the C from the new vegetation can be inferred from the net change in the ^{13}C natural abundance signature of the soil. The turnover of the original vegetation C is calculated using first-order decay model as:

$$MRT = \frac{1}{k} = \frac{t}{\ln(S_t S_o)} \qquad (12)$$

where k is the decomposition constant or turnover, t is the time since conversion, S_t is the C content from the original vegetation at time t, and S_o is the C content at t = 0 [22, 41]. The k value for decomposition or turnover can be calculated as:

$$k = \frac{\ln(S_t / S_o)}{t} \qquad (13)$$

Compared with tracer techniques, the ^{13}C natural abundance technique provides *in situ* labelling of plants and all SOM fractions from relatively short periods (e.g. 5 to 10 years) to thousands of years, including those with extremely long turnover time while the tracer technique only labels SOM fractions for a relatively short turnover time (e.g. days) during the course of the experiment.

However, a major limitation of the ^{13}C natural abundance method is the requirement of soil-plant pairs, which may be unnatural. A maximum range of only 14‰ is available for all variations in $^{13}C/^{12}C$ ratio [45]. The variability of $\delta^{13}C$ value in soil and plant is about ± 1‰ to 2‰ [48]. Thus, a high-resolution and highly sensitive mass spectrometer is required for ^{13}C analysis. Furthermore, the issue of isotopic fractionation is critical for all ^{13}C related methods. Other workers reported isotopic fractionation occurring during lignin synthesis (e.g. [42, 48, 50] and during the decomposition of some organic materials such as roots [27]. The

fundamental differences in $\delta^{13}C$ values exist between soil types [51]. Thus, these factors need to be considered before adopting the use of ^{13}C natural abundance method for general use in soil C sequestration.

2.3 Carbon-14 dating technique

This technique is based on using ^{14}C, the radioactive isotope of C, which has a half-life of about 5,730 years. This ^{14}C is present in plants and its transformation into SOM facilitates the age of the SOM to be determined [51]. Most of the C on Earth exists as ^{12}C. It has been estimated that there is 1 atom of ^{14}C in about 10^{12} atoms of ^{12}C. The ratio of $^{12}C/^{14}C$ on Earth is nearly constant, although some changes occurred in ^{14}C concentration over the last 10 000 years and corrections are made to overcome these systematic errors using international reference standards, corrected to 1950, such as oxalic acid standard, SRM 4990C (U.S. National Bureau of Standards; [53], cockle shells [54] or tree rings [55]. Due to mass differences in C isotopes (^{14}C, ^{13}C, ^{12}C), isotopic fractionation occurs during photosynthesis and in other physical, chemical and biological processes. This is corrected by normalizing to a standard $^{13}C/^{12}C$ ratio, using Equation 11 and PDB limestone as the standard as described earlier.

Carbon-14 in the bodies of plants and animals is constantly being replaced as the plant or animal consume more ^{14}C from the air or through its food. When a plant or animal dies, it stops bringing in new ^{14}C. The ^{14}C already in the plant continues to decay at a constant rate, while that of ^{12}C remains constant. By comparing the $^{14}C/^{12}C$ ratio in the dead plant material to that in the living plant, the age of the dead plant material is determined. As the dead plant material when added to soil is converted to SOM, the degree to which $^{12}C/^{14}C$ ratio in SOM differs from that of the plant material from which it is derived reflects the age of C in soils ([52]. This is often used to calculate the MRT [56–57] and the decomposition rate k according to Equation 5.

A formula used in C dating is

$$t = [\ln (N_f/N_o) / (-0.693)] \times t_{½} \qquad (14)$$

where N_f = percent ^{14}C in the sample

N_o = percent ^{14}C in the living tissue

$t_{½}$ = half-life of ^{14}C (5,700 years)

t = age of sample dated.

Due to the short half-life of ^{14}C, levels of ^{14}C become difficult to measure and compare after 50 000 years (between 8 and 9 half-lives; where 1 percent of the original ^{14}C remains undecayed). Thus, the timeframe of the ^{14}C technique is from 200 to 40 000 years before present (1950).

Because of the low ^{14}C activity present in biological materials submitted for ^{14}C dating, extremely sensitive equipment is required to detect and count ^{14}C emissions. In practice ^{14}C measurements are conducted by specialists in ^{14}C dating laboratories [52, 58]. Several radiation-counting instruments (e.g. gas counters, scintillation counters, accelerator mass spectrometer or AMS) are used to measure the radioactivity of samples containing ^{14}C. The AMS has greatly reduced some of the difficulties of counting small samples containing very low ^{14}C levels such as archived soil samples or fractions of SOM since milligrams of C in

soils or fractions can be counted [59–60]. Another advantage of AMS is the high rate of sample throughput (e.g. 100 samples in 24-hour period).

Carbon-14 dating data are generally modelled to estimate turnover rates of SOM (e.g. [61–65]) based on $\Delta^{14}C$ values using the box model approach [61–65]. The $\Delta^{14}C$ value is defined as:

$$\Delta^{14}C = [(^{14}C/^{12}C)_{sample} / (^{14}C/^{12}C)_{standard} - 1] \times 1000 \qquad (15)$$

expressed in parts per thousand of the $^{14}C/^{12}C$ ratio in the sample to the absolute standard of oxalic acid, as presented earlier [53]. The box model approach partitions SOM into two fractions: labile and refractory or passive pools (Figure 2; [61]). The model assumes that the labile pool has an annual decadal resistance time, while the passive pool has millennial turnover times. Both pools are assumed to have reached a steady state (i.e. inputs = outputs). The time-dependent model is used to calculate the mean turnover time of C (1/k) from the radiocarbon data, assuming that the decomposition of SOM is represented as a first-order process [61–62], as given earlier in Equation 4.

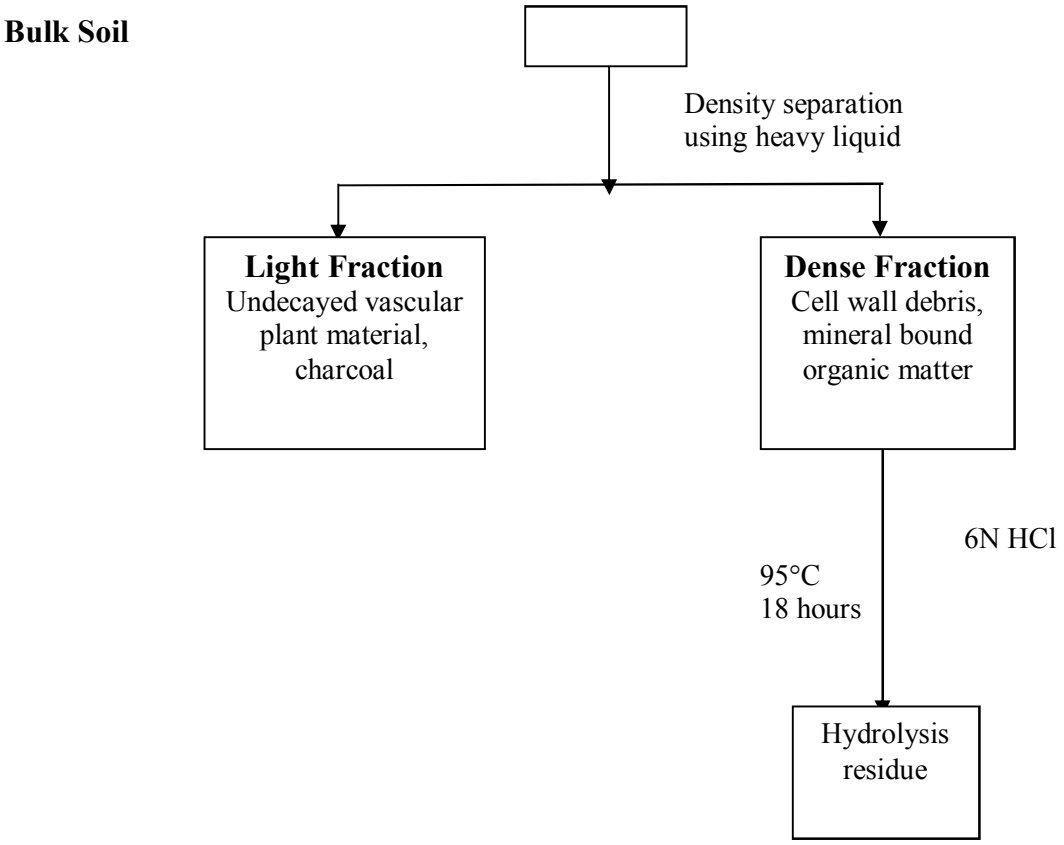

FIG. 2. Fractionation scheme used for soil organic matter in the box model of [61] (Labile SOM = light fraction + dense fraction; refractory or inert SOM = acid hydrolysis residue).

The MRT of soil C is calculated from the box model using a decay constant derived from the ^{14}C content of pre-artificial radiocarbon OM at a steady state [61]) as:

$$R = \frac{r_d}{(r_d + r_{14_C})} \qquad (16)$$

where $R = \Delta^{14}C/1000 + 1$, r_d = decay constant for decomposition (first order), $r_{14_C} = {}^{14}C$ radioisotope decay [61].

The MRT for SOM is $\frac{1}{r_d}$ [56] and r_d value is obtained when R is measured.

Turnover times derived from radiocarbon data in physically and chemically fractionated SOM for the boreal, temperate and tropical soils are given in Table 2. These turnover times vary with soil depths, soil and forest types, SOM fractions and environment. Fractions with most rapid turnover times (years to decades) are the least degraded OM (undecomposed leaves, roots and moss detritus).

TABLE 2. SUMMARY OF TURNOVER TIMES DERIVED FROM MODELLING RADIOCARBON IN SOIL ORGANIC MATTER FRACTIONS FOR THE BOREAL, TEMPERATE AND TROPICAL FOREST SOILS.

Site and SOM fraction	C stock ($g\ C\ m^2$)	TT* (yr)	Source of mean residence time (MRT)
Boreal			
Surface moss and detritus	5800	60	^{14}C and C accumulation since last fire; chronosequence
Humic layer	9400	1000–1500	^{14}C and C accumulation since deglaciation
Total to mineral (40 cm)	15 200	650–1250	
Temperate			
O leaves + roots	400	3–8	^{14}C of leaf and root detritus
O humics	1300	30–40	^{14}C and C accumulated since reforestation; CO_2 fluxes
A/Ap low density roots	100	3–8	^{14}C of root detritus
A/Ap low density humics	2600	50–160	^{14}C of <2 g cm^{-3} fraction
A/Ap dense	600	160–400	^{14}C of >2 g cm^{-3} fraction
B1 low density	1200	800–1000	^{14}C of <2 g cm^{-3} fraction
Total to 40 cm	6200	200–310	
Tropical			
O leaves	325	<1	litter flux and layer inventory
A (0–40 cm) low density	830	1–3	^{14}C of <2 g cm^{-3} fraction
A dense hydrolysable	3110	10–30	^{14}C and C removed hydrolysis
A dense nonhydrolyzable	1190	>6000	^{14}C of >2 g cm^{-3} residue
Total to 40 cm	5460	1040	

**Turnover time of C in the plant + soil system. For a homogeneous, steady-state system, this is the same as the mean age of C in the fraction, or the mean time a C atom has resided in the SOM fraction since it was fixed from the atmosphere by photosynthesis.[62]*

The MRTs of this detrital C pool increases from tropical to boreal latitudes. Fractions with slowest turnover times (centuries to millennia) are either those of SOM associated with the mineral (clay) surfaces or kept from decomposition due to waterlogging or soil freezing (boreal soil) conditions [49, 62]. The mean soil C residence time to 40 cm soil depth

(interpreted as the bulk SOM) ranges from 200 to 1300 years. This age is highly affected by the amount of persistent (millennial cycling) C in the soil. Using the mean ages derived from the bulk SOM radiocarbon measurements would lead to severe underestimation of the dynamic nature of C in these soils, which contain more than 40% of their SOM in forms that cycle on decadal or shorter timeframes [62]. Thus, the use of a single decomposition rate (k) for the bulk SOM may not be valid as SOM is heterogeneous and not all fractions decompose at the same rate.

2.4. Artificial radiocarbon technique

Artificial radiocarbon (AR) to the atmosphere as a result of nuclear weapons testing in the 1950s and 1960s, have resulted in the enrichment of the atmosphere with artificial radiocarbon. Maximum enrichment occurred in 1961 and 1962 accounting for more than 70% of the total (AR) [66]. The enrichment has ceased at present and is depleting annually at about 6.1% per year.

Many studies showed that AR represents a spike input of *in situ* incorporation (i.e. tracer experiment) of artificial radiocarbon -produced into the terrestrial ecosystem through plants and recycled through animals, micro-organisms, soils and SOM (e.g. [50, 61–62, 67–72]. After the nuclear tests stopped, it allows the estimate of the turnover of SOM.

Using Equation 15, $\Delta^{14}C$ values are calculated, with all samples corrected for mass-dependent fractionation of C isotopes, to a common $\delta^{13}C$ value of –25‰, as presented earlier (see Equation 11). Positive $\Delta^{14}C$ values indicate the presence of artificially -produced ^{14}C while negative values indicate that the bulk of C has resided long enough in soils for significant radioactive decay to have occurred. Artificial radiocarbon was a more sensitive indicator of the recent accumulation or loss of C than C inventory measurements using mass balance methods, when comparing soils subjected to different land management practices in eastern Amazonia [73]. Soils with accumulated C during the past 30 years will have more AR than those at steady state, while soils that are losing C will have less AR.

By measuring the AR content of SOM using the same equipment and laboratory facilities as presented earlier for ^{14}C dating [52, 71], the rate of decomposition k can be calculated based on the steady state diffusion model of [75]. A modified version of the diffusion model for calculating the total amount (T_a) of ^{14}C in a soil profile is as follows:

$$T_a = \sum_j l_j P_j C_j \Delta^{14}C'_j \qquad (17)$$

where l_j = depth of horizon j in cm

P_j = soil bulk density in horizon j

C_j = per cent organic C in horizon j

$\Delta^{14}C'$ = increase in ^{14}C in horizon j due to incorporation of AR

Σ = summation of all horizons that show a significant increase in ^{14}C

a = year in which the soil was sampled.

The total amount of organic C per unit area in the soil profile (W) is given by:

$$W = \sum_j l_j P_j C_j \qquad (18)$$

The rate of decomposition k is given by:

$$k = \frac{1}{\tau} \quad (19)$$

where τ = turnover time, estimated by:

$$\tau = W/I \quad (20)$$

where I = annual input of C.

The I value is obtained by measuring the annual amount of plant residues returning to the soil (e.g. [74]. Other measurements (e.g. root growth, soil bulk density, content, etc.) are described [71].

The model (Equation 17) [70, 75] assumes the following:

- A fixed and steady rate of fresh C input as plant residues, which have the same specific activity as that of the atmospheric CO_2.
- The loss of C is by respiration, not distinguishable by ^{14}C loss due to ^{14}C decay.
- The decomposition rate of litter C and of soil C is proportional to their respective C content (first order kinetics as shown in Equation 4).
- There is no isotopic fractionation within the system by physical, chemical or biological processes.
- All vertical movement of C down the soil profile is by diffusion with a constant mean diffusivity over each depth increment.
- The distribution of the 'modern C' which falls with soil depth is in a steady state while that of the 'old' C is more or less constant throughout the soil profile.

These assumptions can be verified by ^{14}C date from a soil profile that has not been exposed to AR enrichment, and is of the same type, preferably occurring adjacent to the site of interest and beneath a structure (e.g. house built before 1950; [76]). Archived pre-artificial radiocarbon soil samples (sampled prior to 1960) or the deepest soil horizon of an exposed soil profile which has not been contaminated with AR can also be used for verification [61, 77]. A comparison of ^{14}C in the pre-artificial radiocarbon and post-artificial radiocarbon soils provides the best means of verifying the assumptions made in the model.

2.5 Soil organic matter models

Data obtained from tracer experiments are often used to develop SOM simulation models. In addition, many models have been established based on theoretical concepts and these models are then verified using data from soil C measurements or using tracer and other C isotopic techniques. In simulation models, the turnover or decomposition rate, k, can be derived or calculated. When these derived k values are applied to Equation 1, soil C sequestration can then be calculated.

An example of using ^{14}C date to develop a SOM simulation model are the experiments conducted and described earlier [38, 40]. Uniformly ^{14}C-labelled ryegrass (*Lolium hybridum*

Hausskn) herbage was added to soils of different mineralogy in micro-lysimeters under field conditions for 5 years. The C data obtained was used to develop a kinetic SOM model by dividing the system into three homogeneous pools or compartments (substrate, biomass, humus) as shown in Figure 3. Microbial biomass and residual ^{14}C data were used simultaneously to estimate mean and variances of residence times in the microbial biomass compartment and in the total system.

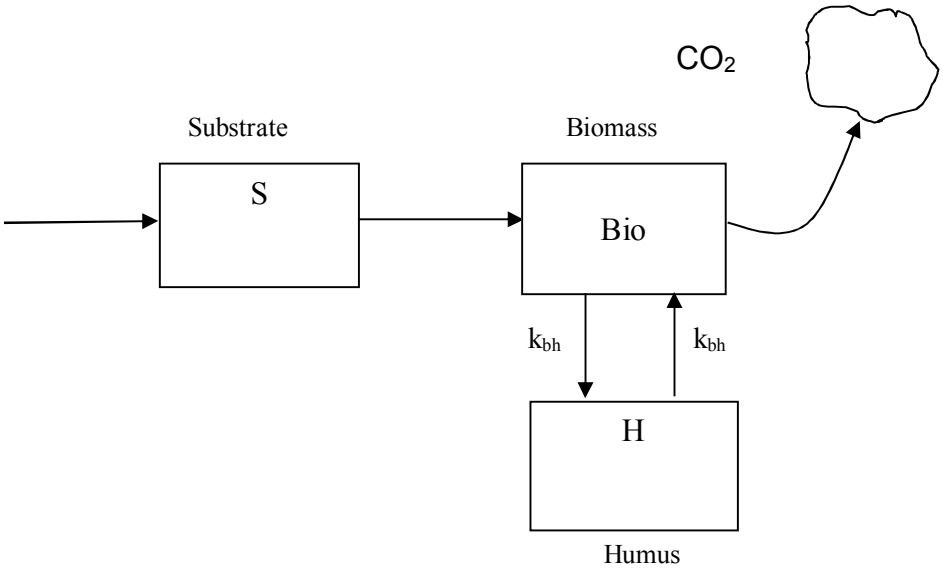

FIG. 3. Schematic representation of the three-compartment model used to estimate the mean residence times of ^{14}C in the soil, microbial biomass and humus. Parameters, k_s, k_b, K_{bh}, represent the first order transfer coefficients. Source: [40].

The equation describing the system is expressed in a matrix form, formulated by assuming first-order kinetics as:

$$\frac{dx}{dt} = Ax \tag{21}$$

$$y = Cx \tag{22}$$

where
$$x = \begin{Bmatrix} S \\ BIO \\ H \\ CO_2 \end{Bmatrix} \tag{23}$$

and y is the vector of the measurable variables BIO (^{14}C-biomass) and CO_2 ($^{14}CO_2$-respired), S, the substrate, represented by amount of ^{14}C-labelled ryegrass C added to each soil and H is the amount of ^{14}C remaining. The coefficient matrices A and C are given by:

$$A = \begin{Bmatrix} -k_s & 0 & 0 & 0 \\ k_s & -(k_{bh} + k_b) & k_{hb} & 0 \\ 0 & k_{bh} & -k_{hb} & 0 \\ 0 & k_b & 0 & 0 \end{Bmatrix} \tag{24}$$

$$C = \begin{Bmatrix} 0\,1\,0\,0 \\ 0\,0\,0\,1 \end{Bmatrix} \tag{25}$$

with the initial conditions of S(0) = So, BIO(0) = O, H(0) = O, $CO_2(0)$ = O.

The first order transfer rates are k_s, k_b, k_{bh}, k_{hb} (Figure 4). The k_s represent the decomposition or turnover rate of the added ^{14}C-ryegrass.

In addition to SOM simulation models based on ^{14}C data in tracer experiments, a number of theoretical models simulating C cycling in soils have been proposed during the last two decades to predict SOC changes with environmental and management practices [78–81]. These models include the Rothamsted ROTHC [82–83], CENTURY [84], APSIM [85], SOCRATES [86] and QSOIL [87] covering time scales of years to a century. Each of the models, except QSOIL, is similar, using a combination of pools with a rapid turnover (annual), moderate turnover (decadal), and slow turnover (millennial) or inert. The QSOIL is a non-compartmental decay model. In all these models, each of the pools is conceptual in nature and generally not measured directly. Details for each of the models cited are available from the references given.

Using the ROTHC model, [88] calculated the decomposition rate constant, k, from Equation 26 based on an exponential decay function as:

$$Y = Yo\ (1 - e^{-abckt}) \qquad (26)$$

where Y is the amount of C that decomposes in a particular month, Yo, the initial amount of C in a particular pool, a, b, and c are the series of modifying factors for temperature, soil water and plant requirements, respectively, affecting C decomposition constant k for that particular compartment, and a constant (t) = $\frac{1}{12}$ to convert k to a monthly time.

Some of the mean residence times obtained in theoretical SOM models and various measured soil physical fractions are shown in Table 3. These MRTs can be used to determine the decomposition rate, k, according to Equation 5. The k values obtained can be used to estimate soil C sequestration using Equation 1.

TABLE 3. MEAN RESIDENCE TIMES (MRT) OF C IN THEORETICAL POOLS OF SOIL ORGANIC MATTER AND MEASURED SOIL PHYSICAL FRACTIONS (ADAPTED FROM [89].

Pool	[90] (Theoretical pools)	[91] (Theoretical pools)	[89]– measured physical fractions
I	Decomposable plant material, 0.24 yr	Metabolic plant residues, 0.5 yr	Vegetative fragments (2–0.2 mm), 0.5–1 yr
II	Resistant plant materials, 3.33 yr	Structural plant residues, 3.0 yr	Vegetative fragments (>0.053 mm) 1–2 yr Vegetative fragments (0.053–0.025 mm), 2–3 yr Macroaggregates (2–1 mm), 1–4 yr
III	Soil biomass, 2.44 yr	Active soil C, 1.5–10 yr	Aggregates (1–0.5 mm), 2–10 yr Aggregates (0.5–0.1 mm), 3–10 years Non-aggregated soil, 7 yr
IV	Physical stabilised, 72 yr	Slow soil C, 25–50 yr	Fine silt (internal), 400 yr
V	Chemically stabilised, 2857 yr	Passive soil C, 1000–1500 yr	Fine clay (internal), 1000 yr

Limited verifications have been conducted on the proposed conceptual SOM models [79]. Comparing 10 SOM models and cited good performance for the ROTHC and CENTURY models, it was reported that using long-term experimental data sets from 7 sites across a range of land uses, soil types and climatic regions found that ROTHC was among the group of 6 models which performed significantly better than another group of 3 models [80]. Comparing the measured and the modelled data for three Australian soil types under two different environments and three different crop rotations using the ROTHC model [88], good agreement between measured and modelled total OC and pool data was observed. This agreement also occurred in Japanese non-volcanic soils [92]. However, in Japanese volcanic soils (Andosols), a good agreement was only obtained between predicted and measured soil C changes after the ROTHC model was modified to cater for the presence of Al-humus complexes causing strong stability in the humus of Andosols [93].

In paddy soils, the DeNitrification-DeComposition (DNDC) model was developed [94] and validated extensively against GHG emissions from agricultural soils in the short-term [95–97]. However, it has not been tested against long-term (i.e. >10 years) changes in SOC content in both upland crop fields and paddy fields. Using adjusted crop parameters, it was found the DNDC model was effective in simulating long-term (16–22 years) SOC dynamics [96] and turnover in paddy soils, except in soils with very low SOC concentration (9.0 g C kg^{-1} soil). Thus, further validating and testing of the accuracy of the conceptualised simulation SOM models with field-derived data are needed.

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CARBON ISOTOPE LABELLING PROCEDURES: TECHNIQUES AND SAFETY ISSUES

J.J. ADU-GYAMFI
Soil and Water Management and Crop Nutrition Section
Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture
IAEA, Vienna
Austria

F. Zapata
Consultant, Soil and Water Management and Crop Nutrition Section
Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture
IAEA, Vienna
Austria

Abstract

The application of radioisotopes as tracers in agricultural research requires suitable facilities including laboratory and measuring/monitoring equipment and adequate training of the personnel to handle these nuclides safely, ensuring that radiation workers are not exposed to undue external or internal radiation hazards. Three tracer techniques and procedures involving ^{14}C or ^{13}C (continuous labelling, pulse labelling and natural labelling based on ^{13}C natural abundance or artificial radiocarbon (AR) enrichment commonly used to determine and estimate plant C inputs into the soil are described. The last part of the paper examines the operational personal safety practices that ensure adequate radiation protection of the workers.

1. LABELLING TECHNIQUES

1.1 Introduction

Three tracer techniques involving ^{14}C or ^{13}C are commonly used to determine and estimate plant C inputs into the soil. These are: (1) continuous labelling, (2) pulse labelling and (3) natural labelling based on ^{13}C natural abundance or AR enrichment.

The first two methods are based on the artificial labelling of whole plant or parts of plant (e.g. a shoot or leaf). This involves exposing the plant or plant part to an atmosphere labelled with $^{14}CO_2$ or $^{13}CO_2$ in an enclosed exposure chamber. Depending on the objectives of the experiments (e.g. following photosynthetic processes, allocation of photosynthetic C to plant roots, rhizosphere, root exudates, etc.) there are innumerable variations on this general theme. For example, the exposure chamber varies from complicated custom-built hemispherical cellulose acetate-butyrate canopy (Figure 1 [1]), aluminium and PVC frames covered with ethyl-vinyl alcohol film (Figure 2, [2]), or a simple chamber to enclose a leaf or leaves with a side-arm reaction chamber (Figure 3).

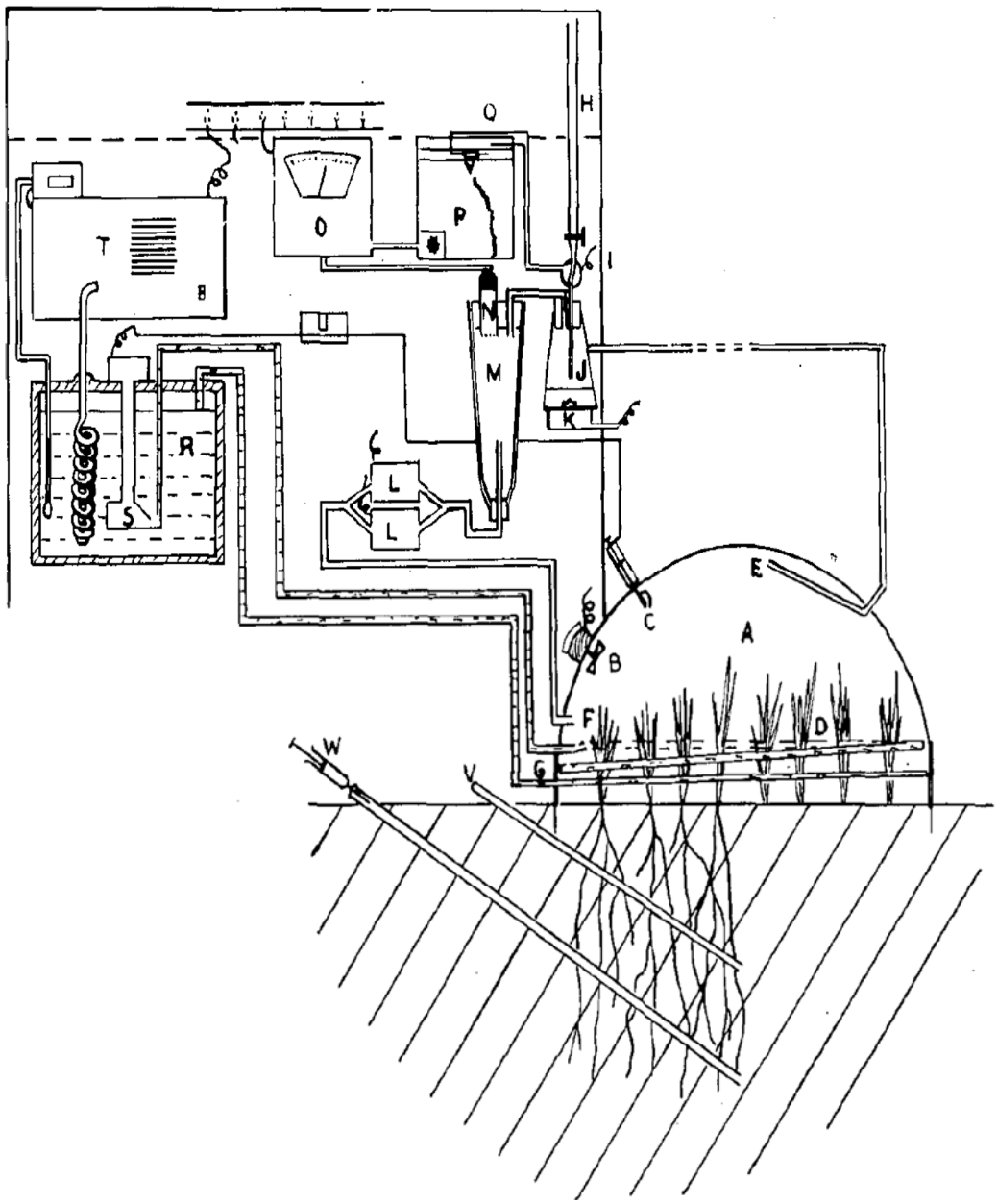

FIG. 1. Growth chamber used for producing labelled plant material by continuous ^{14}C labelling; A = canopy; B = fan; C = thermistor; D = cooling coil; E = air inlet; F = air outlet; G = refrigerant inlet and outlet; H = labelled sodium carbonate inlet; I = magnetic valve; J = lactic acid; K = stirrer; L = pump; M = counting chamber; N = GM counter; O = rate meter; P = recorder; Q = valve switch; R = refrigerant liquid; S = pump; T = immersion cooler; U = temperature control; V = sampling tube; W = disposable syringe [1].

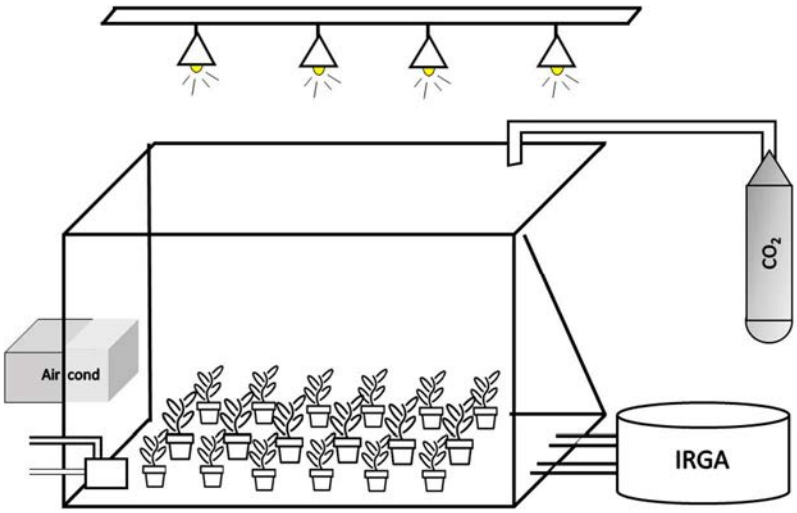

FIG. 2. Labelling chamber for producing labelled plant material by continuous ^{14}C-labelling; IRGA = infrared gas analyser [2].

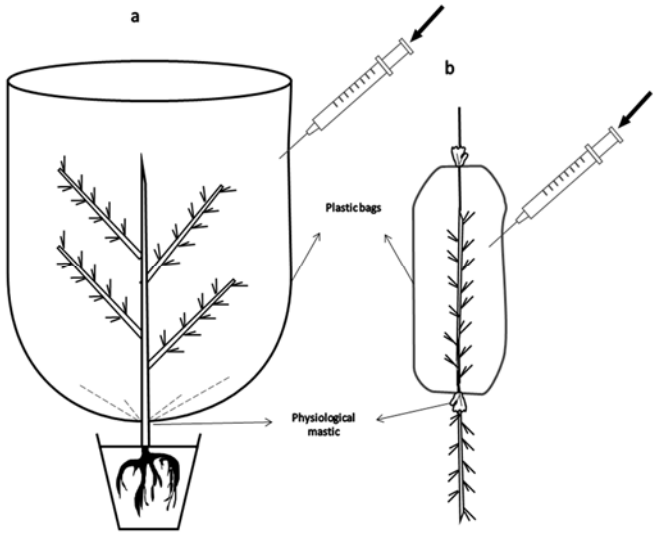

FIG. 3. Flexible exposure chambers for labelling plants or plant parts with $^{14}CO_2$ shown as (a) and (b) [4].

In general, four basic features of labelling equipment are common among the various techniques used. These are: (1) an exposure chamber, (2) a system of delivering $^{14}CO_2$ or $^{13}CO_2$ in order to provide sufficient ^{14}C or ^{13}C at a rate for normal plant C uptake and allowing later measurements of ^{14}C or ^{13}C in the plant, (3) a sample procedure and sample preparation method designed to prevent any loss (e.g. by respiration) in continuous labelling technique, and (4) convenient sample analysis facilities for C and radioactivity measurements [4].

The continuous labelling technique requires specialised growth chambers as described above (Figure 1 and 2) to continuously label the plant for a fixed period to produce uniformly

labelled plants or plant parts. The generation and regulation of $^{14}CO_2$ or $^{13}CO_2$ must be controlled as well as the temperature and moisture conditions inside the chamber. These growth chambers are generally too cumbersome and expensive for field experiments [5–6]. In recent years, there is a general trend towards the use of a simple alternative especially for field experiments such as the ^{14}C or ^{13}C pulse labelling technique. In this technique, the plants or shoots are exposed to the labelled CO_2 only for a short period (0.2–2 hours) by exposing the plants to a large single or repeated $^{14}CO_2$ or $^{13}CO_2$ pulses [7–9]. The quantity of $^{14}CO_2$ (Q : Bq ^{14}C; Bq = 1 disintegrations per second or 37,000 Bq = 1 μCi) used to label the plant material depends on: (a) the desired final specific activity of the labelled plant residues (SA : Bq ^{14}C/g C); (b) the efficiency of the labelling technique for incorporating labelled C into the plant tissue (E : 50–80%); (c) the loss of fixed labelled C through subsequent plant respiration (R: 30–60%) and total plant production at harvest (Y_c : gC) as expressed below:

$$Q = \frac{SA}{ExR} \text{ x } Y_c \qquad (1)$$

The fraction of total $^{14}CO_2$ exposed at any one pulse should be proportional to the rate of growth of the plant biomass production at the time of the pulse [7]. This quantity (Δ : Bq) of $^{14}CO_2$ is:

$$\Delta = Q \text{ x } \frac{\Delta Y_c}{Y_c} \qquad (2)$$

The plant dry matter accumulation over time is estimated from previous years' data, approximations from past experiments or computer simulation of plant growth. In the case of $^{13}CO_2$ pulse, the quantity of $^{13}CO_2$ added during labelling should be sufficient to label the plants at about 500 ‰ $\delta^{13}C$, assuming 50% plant recovery of the added label [3].

Pulse labelling using C isotopes is a useful technique for determining C fluxes and root turnover in pasture species in the field. However, there is a fundamental difference between continuous and pulse labelling [5, 10]. Continuous labelling homogeneously labels the plant while pulse labelling labels the labile plant C pools (i.e. non-structural) and no pool will be labelled homogeneously [11]. Thus, continuous labelling provides information on total C inputs into soil from plant roots while pulse labelling is a useful tool for following C fluxes with changing environmental conditions [6].

Both ^{14}C and ^{13}C have been used in labelling plants in continuous and pulse labelling. Both have their advantages and disadvantages and the choice depends on the objective of the experiment and the availability of equipment and sample analysis facilities. The ^{14}C has high sensitivity, lower costs of the purchase and analyses and easier sample preparation. The ^{13}C, on the other hand, is a stable isotope and thus precautions and safety regulations necessary for radioactive isotopes are not required. Furthermore, both total C and ^{13}C measurements can be made together using a mass spectrometer.

The third labelling technique using ^{13}C natural abundance or AR enrichment relies on the natural labelling of the plants in the field without the need of introducing ^{14}C or ^{13}C label artificially into the plants. The ^{13}C natural abundance is based on the discrimination of ^{13}C and ^{12}C during CO_2 assimilation by plants with different photosynthetic pathways [12] while the AR method is based on the natural enrichment of the soil and vegetation in the field with ^{14}C from the detonation of thermonuclear devices in 1950s to 1960s [13–14]. This third method has the principal advantage of determining the plant C input into soil under field conditions without the need of special plant labelling equipment. However, the method

requires a number of conditions (e.g. pair of C_3 and C_4 plants) and assumptions and also the use of models to estimate C input [10, 13, and 15].

Detailed principles and procedures for using ^{13}C natural abundance or AR method for determining soil C sequestration are explained elsewhere (Paper 3). Four procedures (i) Continuous ^{14}C-labelling (ii) Continuous ^{13}C labelling (iii) ^{14}C pulse labelling (iv) ^{13}C pulse labelling have been described elsewhere [2–3, 8–9].

2. SAFETY ISSUES IN HANDLING CARBON ISOTOPES

The application of radioisotopes as tracers in agricultural research requires suitable facilities including laboratory and measuring/monitoring equipment and adequate training of personnel to handle these nuclides safely, and ensuring that radiation workers are not exposed to undue external or internal radiation hazards. Member States are required to establish radiation safety infrastructure to ensure safe use of radioisotopes users of radioactive material should always consider specific national legislation and regulations that control their use.

Occupational exposure to ionizing radiation can occur as a result of various human activities, including the use of radioactive sources in industry, agriculture, medicine and many fields of research, that involve the handling of materials with enhanced concentrations of naturally occurring radionuclides. This is particular the case when working with the most common radiotracer nuclides (β-emitters) used in agricultural research, i.e. ^{32}P (β^-, 1.7 MeV, t/2=14.3d), ^{33}P (β^-, 0.25 MeV, t/2=25.3d), ^{35}S (β^-, 0.17 MeV, t/2=87d) and ^{14}C (β^-, 0.15 MeV, t/2=5730a), that are handled as so called “unsealed sources”.

2.1 IAEA Safety Fundamentals

The fundamental safety objective and associated principles to protect people and environment against harmful effects of ionizing radiation, including the safety of facilities and activities that give rise to radiation risks are presented in the IAEA Safety Standard Series SF -1 [16]. It states ten (10) safety principles: (1) responsibility for safety (2) role of government (3) leadership and management for safety (4) justification of facilities and activities (5) optimization of protection (6) limitations of risk to individuals (7) protection of present and future generations (8) prevention of accidents (9) emergency preparedness and response and (10) protective actions to reduce existing or unregulated radiation risks.

2.2 IAEA Safety Standards

The IAEA Safety Standards Series comprises Safety Fundamentals, Safety Requirements and Safety Guides are binding to the IAEA in its own operations, are applied by other sponsoring organizations for their s own operations and are recommended for use by Member States and national authorities in relation to their own respective activities. These safety standards are not legally binding on Member States, but may be adopted by them, at their own discretion, for use in national regulations with respect to their own activities. Governments, however, have responsibility for establishing a national radiation safety infrastructure in support of their use of applications of ionizing radiation. The IAEA Safety Requirements Series on Governmental, Legal and Regulatory Framework for Safety [17] lists the responsibilities and functions of governments in relation to safety. Other publications in the IAEA Safety Standards Series establish detailed requirements relating to those

responsibilities and address in particular the protection of workers, patients and the public, and the protection of the environment in all exposure situations and in a variety of facilities and activities.

2.3 Operational Radiation Protection and Safety procedures

The IAEA Safety Standards Series No GSR Part 3 on General Safety Requirements entitled 'Radiation Protection and Safety of Radiation Sources: International Basic Safety Standards'[16], is co-sponsored by seven other international organizations European Commission, FAO, ILO, OECD/NEA, PAHO, UNEP and WHO. It details the requirements for the protection of people and the environment from harmful effects of ionizing radiation and for the safety of radiation sources [16]. Requirements established in this publication, as well as specific guidance established in other IAEA publications [17–18] could serve as a basis for the preparation and implementation of local rules and procedures directed to ensure an adequate level of radiation protection of workers and members of the public against the radiation risks associated with the different applications of radiation sources. Several examples [19–21] exist of the applications of radiation protection standards in those facilities and activities dealing with unsealed sources.

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APPENDIX

SIX CASE STUDIES ON THE USE OF ^{13}C TO DETERMINE SOIL CARBON SEQUESTRATION AND ^{14}C FOR CARBON TURNOVER AND DATING

Case Study A. A method for determining soil carbon sequestration using ^{13}C-labelling and an optical breath test analyser

Case Study B. ^{14}C-labelled ryegrass to measure carbon turnover and residence times in soils of different clay content and mineralogy

Case Study C. A chamber method for the in situ labelling of pasture sward to determine carbon inputs and root decomposition

Case Study D. In situ pulse labelling of plants with ^{13}C in the field to examine plant ^{13}C uptake and allocation

Case Study E. Measurement of soil organic carbon turnover using ^{13}C natural abundance

Case Study F. Estimating the turnover of soil organic matter using radiocarbon dating

A. A METHOD FOR DETERMINING SOIL CARBON SEQUESTRATION USING ^{13}C-LABELLING AND AN OPTICAL BREATH TEST ANALYSER

A.1 Background

A variety of techniques involving different C isotopes (e.g. ^{13}C, ^{14}C) have been used to determine the decomposition of SOM C but few attempts have been made to incorporate these data directly for determining soil C sequestration. An optical system (isotope-selective non-dispersive infrared spectrometry) developed by [1–2] was used for breath testing into a robust, low-cost (US$15 000–25 000) FANci2 method for ^{13}C analysis. The equipment requires relatively low maintenance and minimal training. In combination with simple ^{13}C plant-labelling techniques the method can be used to determine soil C sequestrating for estimating C credits. Thus, this system has considerable advantages for measuring soil C sequestration under different agronomic management practices and land use changes within a short time frame, especially in developing countries.

An earlier study [1] compared the measurements of $\delta^{13}C$ values using the FANci breath test analyser and the mass spectrometer. Both natural abundance and enriched native C-3 organic matter and C-4 sugar were used. Significant correlations were obtained between the two methods (r^2 = 0.84 to 0.96). The protocol reported is designed to show the simplicity and reliability of the method.

A.2. Principle of FANci method

The traditional method for determining $^{12}C/^{13}C$ ratio is to use the isotope-ratio mass spectrometer [3]. This instrument is expensive to purchase and maintain. It requires highly trained technical personnel to operate the instrument. The FANci2 system provides the option of an easy to operate, robust and low-cost system employing the optical systems used in breath testing to measure $^{12}C/^{13}C$ ratio [1–2].

The FANci2 (FAN GmbH, Leipzig, Germany) system is basically an infrared gas analyser with two analysis chambers, one with a static reference gas and the other, the sample chamber, where the sample gas is pumped and analysed as in the breath test analysis. The range of detection is about 1.5–5.5% CO_2.

A.2.1 Procedures

Cowpea plants (var. AY1Y1) were grown from seed in PVC tubes containing 3 kg quartz sand in the greenhouse and labelled with ^{13}C using the growth chamber as shown in Figure A.1

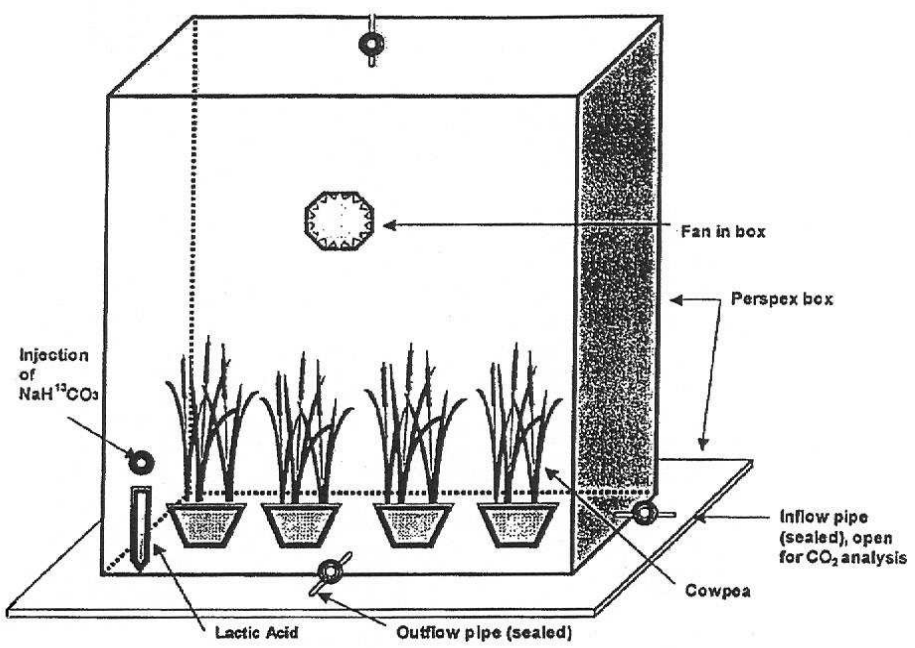

FIG. A.1. Growth chamber used for labelling plants with $^{13}CO_2$.

The seedlings were watered daily with a Long Ashton solution labelled with 5 atom % excess ^{15}N. Three weeks after planting the plants were pulse-labelled with ^{13}C. This was achieved by placing the plants in a gas tight Perspex air-conditioned chamber (Figure A.1), which contained a vial of concentrated lactic acid. The ^{13}C-sodium bicarbonate (99 atom %) solution (0.5 mol/L, 10 mL) was added to 7.5 mol/L lactic acid to release labelled CO_2. Two and four hours after the initial injection, 10 mL unlabelled sodium bicarbonate solution was added to the acid, this ensured most of the labelled CO_2 was taken up by the plants. The CO_2 concentration was monitored throughout 24-h labelling period using a conventional infrared gas-analyser. The labelling procedure was repeated twice weekly for two weeks and the plants harvested 3 days after the last injection. Plant material was separated into shoots and roots, dried at 70°C, and ground for ^{13}C and ^{15}N mass spectrometric analysis (Optima, Micro mass, UK).

A.2.2 Comparison experiment

Materials produced using the above system with a range of ^{13}C values were compared using mass spectrometry and the FANci system. For FANci analysis, plant material was digested [4]. The tubes containing the plant sample, digestion mixture, and CO_2 trap were placed in a heating block for 1 h at 130°C, then left to cool, and left overnight to enable complete CO_2 absorption. The following day the CO_2 traps were removed. An aliquot of NaOH was back-titrated with 0.01mol/L HCl, indicator phenolphthalein (1% in alcohol) after addition of 10 mL 1 mol/L $SrCl_2$. The $SrCO_3$ precipitate was washed and centrifuged three times and then dried at 40°C. The $SrCO_3$ precipitate was analyzed as described above using either the FANci apparatus or the mass spectrometer. Three replicate samples per material were prepared and analyzed.

A.2.3. FANci analysis

Approximately 40 mg $SrCO_3$ was added to a 10 mL Vacutainer which was then evacuated, 1 mL 7.5 mol/L phosphoric acid was added and vigorous effervescence was observed. Using a gas-tight syringe 5 mL CO_2 was sampled from the tube and added to the gas sampling bag through a Suba Seal, an additional 95 mL air was added and the sample bag connected to the FANci breath test analyser (FAN GmbH, Leipzig, Germany). The samples were run using a delta over baseline (DOB) routine using laboratory standard gas (5% CO_2 in air 18‰ $\delta^{13}C$) as the baseline standard.

A.2.4. Carbon sequestration experiment

The aim of the experiment was to establish whether it is possible to undertake carbon sequestration studies using the FANci apparatus. The soil used was a low-carbonate sandy loam soil from the Krumbach region, Austria. There were 12 replicates of each treatment to allow for weekly destructive sampling.

The treatments were:

1. No residue added, soil only control;
2. Cowpea leaf residues added at a rate equivalent to 1134 mg C/kg soil, 100 mg N/kg soil (equivalent to 200 kg N/ha, 2.26 tonnes of carbon per hactare); and
3. Cowpea root residues added at a rate equivalent to 1250 mg C kg^{-1} soil, 100 mg N kg^{-1} soil (equivalent to 200 kg N/ha, 2.5 tonnes of carbon ha^{-1}).

Soil (300 g, dry-weight equivalent) and 50 mL water were mixed according to treatment and packed into PVC incubation tubes. The soil samples were placed in a gas-tight 1.5 L Kilner jar and a CO_2 trap containing 40 mL 0.5 mol/L NaOH was placed on the top of the tube and supported by chicken wire. At weekly intervals for three weeks, the CO_2 trap was replaced and the jar aired. SrCl was added to an aliquot of NaOH and back titrated with HCl and prepared for FANci analysis as described above.

The C derived from the plant residue (%Cdfr) was calculated as:

$$\%\text{Cdfr} = \frac{\delta^{13}C \text{ of respired } CO_2}{\delta^{13}C \text{ of residue to soil}} \text{ x } 100 \qquad (1)$$

A.3. Results and discussions

The ^{13}C labelling of the cowpeas was successful, as shown by the results in Table 1.

TABLE A.1. PLANT QUALITY CHARACTERISTICS OF COWPEA GROWN IN LABELLING CHAMBER.

Treatment	$\delta^{13}C$ ‰	%C
2 Leaves	2130	41.95
3 Roots	2485	38.89

The δ ^{13}C was much higher than background. The uniformity of labelling was examined in a separate experiment by analysing the ^{13}C of the lignin, cellulose, and acid detergent fractions. There were major differences in the enrichments of the fractions (data not shown).

There was a highly significant linear correlation of ^{13}C value measured in plant residues using either the mass spectrometer or digestion procedure and analysis using the FANci (r^2 = 0.99; Figure A.2).

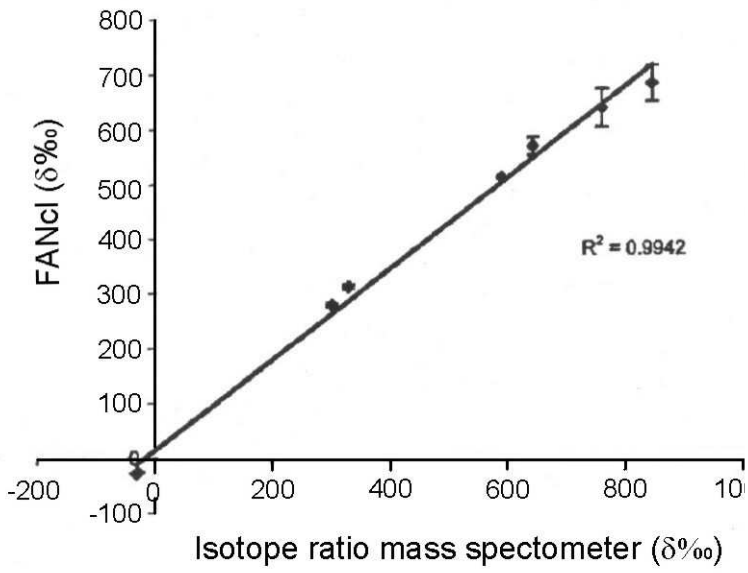

FIG. A.2. Comparison of $^{12}C/^{13}C$ ratios in plant samples measured directly using continuous-flow mass spectrometry or prepared using digestion followed by measurements using the FANci 2 (± deviations).

It was possible to measure C derived from residues using both the isotopic and non-isotopic methods. Both gave similar values suggesting that the techniques for measurement were appropriate (Table A.2). Using these data it was possible to construct rough C budgets for an equivalent inorganic N fertilizer system. The C sequestration was calculated by subtracting the respired C from the original input value using the isotopic data. From this perspective the cowpea residue treatment had significant benefits in terms of C sequestration, if it is assumed that residues are stabilised in the soil. This assumption is not necessarily true, however, using the techniques described and measuring the ^{13}C remaining in the soil it would be possible to quantify this over many years.

The results of this study show that soil C sequestration can be determined using the optical breath test analyser in combination with a ^{13}C-labelling method.

TABLE A.2. PERCENTAGE OF RESIDUE MINERALISED AS CO_2 CALCULATED USING DIFFERENCE APPROACH AND ISOTOPIC APPROACH (EQUATION 40).

Treatment	% of residue added mineralised as CO_2 difference approach			% of residue added mineralised as CO_2 isotopic approach		
	7 days	14 days	21 days	7 days	14 days	21 days
2 Leaves	10.21	2.14	1.51	9.67	1.29	0.24
	(0.06)	(0.1)	(0.01)	(0.15)	(0.11)	(0.02)
3 Roots	5.52	2.04	0.86	4.40	0.30	0.15
	(0.04)	(0.1)	(0.01)	(0.04)	(0.11)	(0.02)

The source of this case study is based on Reference [2].

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B. ^{14}C-LABELLED RYEGRASS TO MEASURE CARBON TURNOVER AND RESIDENCE TIMES IN SOILS OF DIFFERENT CLAY CONTENT AND MINERALOGY

B.1. Background

Uniformly isotope-labelled (^{13}C or ^{14}C) plant residues are commonly used to study the decomposition and turnover rates of C when added to the soil in the field or greenhouse. This study demonstrated the decomposition of uniformly labelled (^{14}C) ryegrass (*Lolium hybridum* Haussukn) added to soils of different clay and mineralogical composition and allowed to decompose under field conditions for five years.

A similar technique for the production of ^{13}C-labelled plant residues has been described earlier (Paper 4). These ^{13}C-labelled plants or residues can also be used to determine decomposition rate, k, the decomposition and soil C sequestration as described here for ^{14}C-labelled plant residues.

The protocol reported here is to show that this technique can be used to determine the decomposition rate, k, of plant residues added to soil under field conditions. The k values obtained can then be used to estimate C loss followed by estimating soil C sequestration based on Equation 1 (Paper 3).

B.2. Procedures

Replicate samples of four soils (100 g oven-dry basis) from sheep grazed pastures with different clay content and mineralogical composition were amended with freeze dried and ground C-labelled ryegrass plants at a rate of 0.5% w/w [1]. The four soils used were Halcombe silt loam, Horotiu silt loam, Hauraki clay and Naiko clay from the central North Island, New Zealand. The uniformly-labelled ryegrass plants were produced in a custom-built Perspex growth chamber based on a design described elsewhere [2–3]. Seeds of ryegrass (*Lolium x hybridum* Hassukn cv. Ariki) were germinated on moist filter paper in warm conditions (8–10 days), then transplanted into a sand-solution system in plastic trays and transferred to the growth chamber. The day (18–20°C) and night (15–18°C) temperatures were maintained by supplementary lighting and a heating and cooling system. Labelled $^{14}CO_2$ was generated *in situ* within the chamber by adding dilute H_2SO_4 to a $Na_2{}^{14}CO_3$ solution. The concentrations and volumes of carbonate and acid were calculated to release 415 mg CO_2, sufficient to increase CO_2 concentration in the chamber by 0.13% (v/v). The CO_2 concentration was monitored at least once daily by gas chromatography and replenished when below ambient level (0.03%) by injecting $Na_2{}^{14}CO_3$ and acid into the chamber. The growth chamber was normally operated for 40–60 days. The plants were harvested when they were about 15–20 cm tall and before anthesis occurred. They were separated into roots and shoots, and freeze-dried. The freeze-dried plant material was ground to <1 mm with a domestic electric coffee grinder and used for the decomposition studies. The C and N contents of the ryegrass were 41.1 and 2.8% respectively, and the ^{14}C specific activity was 4.27 MBq/g C.

The soil samples were well mixed with the ground labelled ryegrass by shaking and stirring, and placed in micro-lysimeter tubes [4]. The tubes were inserted into a pasture site. The lysimeters were capped with mesh to retain contents and periodically cleared of plant growth. The amended soils were left for up to five years under field conditions and destructively sampled (in triplicate) after 0.2, 0.5, 1.0, 2.5, 4.0 and 5.0 years to determine the amount of total ^{14}C remaining and the amount of ^{14}C incorporated into microbial biomass. Each soil sample was divided into three sub-samples. The control (non-fumigated) samples

were extracted immediately in 0.5 M K_2SO_4, the fumigated samples were extracted 24 h later after fumigation with ethanol-free $CHCl_3$, and the remaining sample was dried (50°C, 48–72 h), ground and sub-sampled for organic C and ^{14}C analysis.

B.2.1. Determination of microbial and total soil C and ^{14}C

Fumigated and non-fumigated soils were extracted with 0.5 M K_2SO_4 for 30 min (1:5 soil-to-extractant ratio), filtered, and an aliquot was analysed for organic carbon by the acid-dichromate oxidation. The ^{14}C content of another aliquot was determined by scintillation counting (see below). The additional oxidizable C and ^{14}C counts obtained from the fumigated soils were taken to represent the microbial-C flush and were converted to microbial biomass C using the relationship: microbial C = C-flush/0.35 [5]. A factor of 0.35 was used.

Total C in soils was analysed by oxidation and digestion as described by [3]. The CO_2 evolved during digestion was trapped in a solution of 2M NaOH and 0.2 M Na_2CO_3 [6]. The CO_2 absorbed was measured by titration of an aliquot (0.6 ml) of alkali against 0.1 M HCl to determine the residual alkali, after first precipitating out carbonates by addition of 25 ml 10% $BaCl_2$, with phenolphthalein as indicator. The radioactivity of $^{14}CO_2$ absorbed in alkali was determined on aliquots (0.2 ml) mixed with 5 ml PCS (Amersham International) containing 2 ml water. An LKB 1217 Rackbeta scintillation counter, with standard parameters for ^{14}C counting and automatic quench correction, was used to measure ^{14}C.

Results were expressed on the basis of oven-dry (105°C) weight, unless otherwise stated. All analyses were made in duplicate.

B.2.2 Mathematical modelling

A kinetic model was developed by dividing the system into three homogeneous compartments of 'pools' and formulated by assuming first-order kinetics. The equations that describe this system are given in paper 3 (Equations 21 to 25).

The general solution of a three compartment open system is given by:

$$\sum_{i=1}^{3} a_i e^{\lambda_i l} \qquad (1)$$

where the constants a_i are called pre-exponential constants and λ_I the eigenvalues of the system. The eigenvalues are macro-rate constants of a dynamic system that govern many physical properties of the system. They are roots of a polynomial equation and a function of all or most of the microscopic rate constants.

Two algorithems were used to estimate the parameters k_s, k_{bh}, k_b, k_{hb} (Paper 4 and Figure 3) and derive the analytical solutions from the experimental data. [7–8]. Initial estimates of these parameter values were obtained by exponential peeling [9] and by nonlinear regression. The results of these algorithms were consistent with each other.

The first and second statistical moments for ^{14}C in the system were obtained from these parameter values. For the system (Paper 3, Equations 21 and 22), these are given [10] as:

$$MRT = \frac{1}{k_s} + \frac{1}{k_b}(1 + \frac{k_{bh}}{k_{hb}}) \quad (2)$$

$$VRT = \frac{1}{k_s^2} + (\frac{k_{bh} + k_{hb}}{k_b k_{hb}})^2 + \frac{2k_{bh}}{k_b k_{hb}^2} \quad (3)$$

The MRTs and VRTs (variance in residence time) of ^{14}C for individual compartments may be similarly obtained [11–12]. The system MRT(S) is the sum of individual compartment MRTs.

B.3 Results and discussion

B.3.1. Decomposition of labelled ryegrass

Amounts of labelled C retained by the amended soils from 9 weeks to 5 years are presented in Figure B.1. Over the first 9 weeks labelled ryegrass was rapidly mineralised in all the soils.

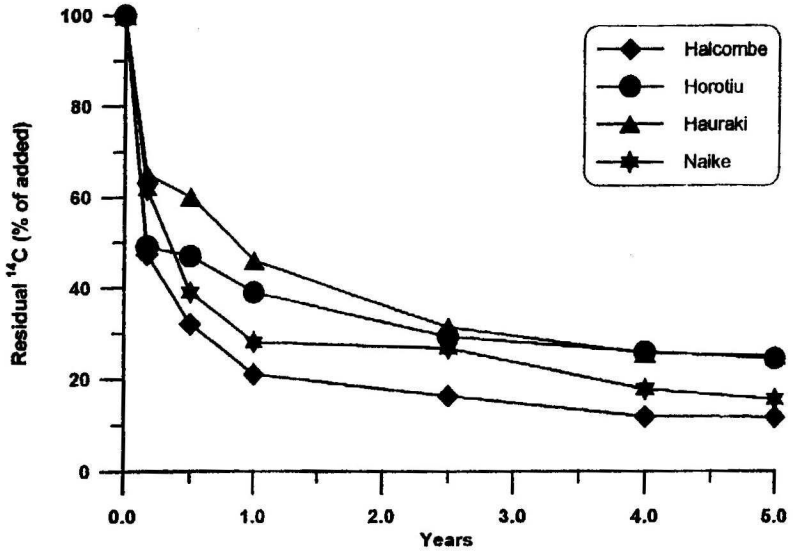

FIG. B.1. Residual ^{14}C from labelled ryegrass decomposition in four soils varying in clay content and clay mineralogy.

Subsequent decomposition was slow. About 35–52% of the labelled ryegrass C was lost in the first 9 weeks, after which it took nearly 5 years to lose another 25–46% labelled C.

B.3.2. Microbial biomass ^{14}C

In each of the soils, the microbial biomass ^{14}C was greatest at day 63 of the incubation and declined during the 5 year incubation (Table B.1). In the later stages of decomposition (years 1–5) microbial biomass ^{14}C was 0.4–3.5% of ^{14}C added.

TABLE B.1. ^{14}C-MICROBIAL BIOMASS AT DIFFERENT STAGES OF DECOMPOSITION IN FOUR SOILS AMENDED WITH ^{14}C RYEGRASS.

Soil	^{14}C-microbial biomass (Bq/g soil) at day					
	63	183	365	912	1460	1825
Halcombe	77 ± 56	331 ± 14	187 ± 12	47 ± 8	39 ± 2	30 ± 4
Horotiu	457 ± 35	410 ± 18	400 ± 21	162 ± 16	73 ± 3	91 ± 5
Naike	806 ± 68	480 ± 21	728 ± 42	333 ± 22	193 ± 30	153 ± 3
Hauraki	641 ± 56	391 ± 16	375 ± 34	211 ± 14	86 ± 6	78 ± 12

B.3.3 Changes in residual carbon

The amount and type of clay was significantly related to the amount of ^{14}C remaining in the four soils (Table B.1). After 9 weeks of decomposition 47 and 49% residual ^{14}C was found in the silt-loam soils (Halcombe and Horotiu soils with 24 and 16% clay content, respectively). This compared with 62 and 65% residual ^{14}C in the clay soils (Naike and Hauraki soils with 56 and 60% clay content, respectively), suggesting that the initial decomposition was slowed by the amount of clay. However, after 6 months of decomposition, 33% of residual ^{14}C was found in the vermiculitic Halcombe soil compared with 46% in the amorphic Horotiu soil. Similarly, of the two clay soils, 38% residual ^{14}C was found in the kandic Naike soil compared with 59% in the smectitic Hauraki soil. This trend continued for the remaining 4.5 years' of incubation.

The rate constants (k) for decline of residual organic ^{14}C, at different periods of incubation, were also calculated using the Equation 4, given [12] as:

$$k = -\ln \frac{(^{14}C_{resid})t_2}{(^{14}C_{resid})t_1} (t_2 - t_1) \qquad (4)$$

where $(^{14}C_{resid})t_1$ and $(^{14}C_{resid})t_2$ are the residual ^{14}C contents at times t_1 and t_2, respectively.

For each soil, the decomposition rate constant (k) declined with the duration of incubation (Table B.2).

TABLE B.2. DECOMPOSITION RATE CONSTANTS (K) OF ORGANIC ^{14}C IN FOUR SOILS AMENDED WITH ^{14}C RYEGRASS.

Soil	k x 10^{-4} (day^{-1}) between days					
	0–63	63–183	184–365	365–912	913–1460	1461–1825
Halcombe	118	30	25	4	6	<1
Horotiu	113	5	11	5	2	2
Naike	77	40	19	4	4	4
Hauraki	69	8	16	6	4	<1

B.3.4. Mean residence time

The main residence times (MRTs) and the variances (VRTs) for the whole system and the MRTs for the three compartments of the system (added ryegrass, microbial biomass and humus) are presented in Table B.3. The coefficients of variation (derived from VRT, Equation 3) of the system residence times were between 95 and 102%. The soils differed widely in system residence times. There was no consistent trend in these values with respect to clay content.

TABLE B. 3. ESTIMATED MEAN AND VARIANCES OF RESIDENCE TIMES FOR THE THREE COMPARTMENT MODEL .

Soil	Mean residence time (MRT) (years)			Variance residence time (VRT) (years)	
	System	Ryegrass substrate	Microbial biomass	Humus	System
Halcombe	1.77	0.22	0.08	1.47	3.28
Horotiu	3.65	0.21	0.12	3.32	6.64
Naike	2.50	0.17	0.13	2.20	13.08
Hauraki	3.91	0.42	0.14	3.36	13.73

There was no positive correlation between the MRT(S) and clay content (expressed as %w/w) of these four soils. This residence time, however, was directly related to the surface area as:

$$\text{MRT (years)} = 1.05 + 0.027 \text{ x (surface area)} \quad (5)$$

This relationship was highly significant ($P < 0.001$) and accounted for 98.9% of the variation in system (MRTs in these soils). Equation 5 indicates that with each 10 m^2 increase in specific surface area the added substrate would remain ca. 3 months longer in the soil.

This study shows that the extent to which microbial metabolites produced during the decomposition of labelled ryegrass are stabilised depends on the nature of the clay minerals present rather than on their amount. With increased surface area more organic residues accumulate and their decomposition is slowed down. In soils of similar texture (clay content) more ^{14}C was retained by smectitic and allophonic soils than by their vermiculitic and kandic counterparts. This study has highlighted the importance of surface area in controlling the decomposition of ^{14}C-labelled ryegrass. It provided estimated values for the decomposition rate, k, which can be used to calculate soil C sequestration using Equation 1, Paper 3.

The source of this case study is based on Reference [1]

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C. A CHAMBER METHOD FOR THE IN SITU LABELLING OF PASTURE SWARD TO DETERMINE CARBON INPUTS AND ROOT DECOMPOSITION

C.1. Background

A simple chamber technique is described for the in situ labelling of pasture swards in the field using ^{14}C pulse labelling by exposing the plants to a large ^{14}C-CO_2 pulse. The feasibility of this technique is presented. A subsequent field study using this technique for investigating seasonal and annual inputs of C and root decomposition is also presented.

The protocol presented here is to show the application of a simple field labelling technique to follow C input in plants followed by the determination of the rate of decomposition of the incorporated C in the roots in the soil. The decomposition or turnover rate, k, as obtained can then be used to estimate C loss followed by estimating soil C sequestration.

C.2. Evaluation of the in situ field labelling technique

C.2.1. Procedures

C.2.1.1 Labelling chamber and technique

A hemispherical chamber made from a specially adapted fishbowl was used as the labelling chamber. The fishbowl (9 litre capacity) was attached to a half-turn locking PVC 'Sewer-hatch' rim (300 mm) using an industrial silicone caulking material and screws. The base of the PVC rim was attached on to a (100 mm deep and 250 mm diam) PVC pipe (lysimeter). The PVC rim and lysimeter attachment had an internal half-turn locking system and a rubber ring to form an air tight chamber inside the fishbowl-chamber above the soil surface. Each bowl was fitted with two rubber septums to allow injection of ^{14}C-CO_2 gas and collection of air samples.

The pasture was mowed 2–3 days before ^{14}C-labelling to leave about 20 mm stubble. The PVC pipes (lysimeters) were slowly driven into the pasture soil. To minimise the friction between soil and lysimeter wall, the lower end of the lysimeter was ground to provide a sharp edge. About 10 mm of the lysimeter's upper end was left above the soil surface. On the labelling day, the hemispherical chamber was attached to each lysimeter with the rubber 'O' ring forming a gas tight seal. The ^{14}C-CO_2 gas was produced in a 60-mL plastic syringe fitted with a 3-way stopcock. Ten mL of ^{14}C-sodium bicarbonate (Na_2CO_3) was sucked into the syringe and neutralised in the body of the syringe with 5 mL M sulphuric acid (H_2SO_4) by manipulation of the stopcock. Once evolution of CO_2 had ceased, the liquid was eluted and the ^{14}C-CO_2 remaining injected into the bowl through the rubber septum through a hypodermic needle.

Total CO_2 and ^{14}C-CO_2 were monitored with time to check the assimilation of the labelled CO_2 by the pasture. Periodically air samples (3 mL) taken from each chamber were absorbed in 3 mL 2M sodium hydroxide (NaOH) + 0.2M Na_2CO_3. The ^{14}C was estimated using a scintillation counter and total CO_2 by gas chromatography. After assimilation of the labelled C by the plant, the pasture was opened to the natural atmosphere and conditions. The allocation period after which the labelled pasture was sampled was between four hours and 35 days.

C.2.1.2. Sampling and analysis

Six cores (50 mm diameter and 100 mm deep) including the herbage were taken at 7 and 35 day (after pulse labelling) intervals. The above ground parts of the labelled plants were clipped. Roots were then carefully washed in distilled water from one half of the cores. Visual observation was used to separate the roots. The dead dark brown/black material was discarded but any stem or stolons present in the soil were included. The remaining cores were sieved for total C and ^{14}C analyses. Samples of the above-ground herbage, root material, and sieved soil were oven-dried and herbage and root material weighed. All the samples were finely ground to <0.2 mm with a domestic electric coffee grinder in a fume cupboard.

In another experiment, triplicate turves maintained at 350 ppmv and 700 ppmv CO_2 levels were ^{14}C labelled over a period of two days. Full details of the study site, turves, CO_2 treatments, pasture production, and composition and ^{14}C flows have been reported elsewhere [1–3]. Roots, herbage and soil collected from this experiment were separated and analysed as described above.

Total C in soils and herbage was analysed by oxidation and digestion. A modified digestion-tube apparatus incorporating a CO_2 trap was used as described by [4]. The digestion mixture comprised 600 mL conc. H_2SO_4, 400 mL 80% phosphoric acid (H_3PO_4), and 100 g potassium dichromate ($K_2Cr_2O_7$). The CO_2 evolved during digestion was trapped in a solution of 2M NaOH and 0.2M Na_2CO_3 [5]. An aliquot (0.2 mL) was taken for liquid scintillation counting and another aliquot (0.6 mL) for titration as above.

C.3. Results and discussion

As shown in Figure C.1, the ^{14}C-CO_2 levels decreased to <0.5% with two hours. The ^{14}C-CO_2 levels in all the three replicates maintained at ambient and elevated CO_2 levels and pulse labelled between 900 and 1300 hours over the period of two days showed a similar quick decline suggesting assimilation of ^{14}C by the pasture sward was not influenced by either the time of the day or initial CO_2 level.

Up to 78% of the calculated ^{14}C-CO_2 produced in the syringe and injected into the chamber was accounted for in shoots, roots, and soil, four hours after labelling. The ^{14}C-CO_2 was produced in a syringe by reacting ^{14}C-Na_2CO_3 with H_2SO_4, and the resultant Na_2SO_4solution was eluted before ^{14}C-CO_2 was injected. A substantial portion of the ^{14}C-CO_2 (>20%) escaped between its production in the syringe and a short assimilation period. Air samples collected around the outside of the chamber during the assimilation period did not contain ^{14}C-CO_2 suggesting that the unaccounted ^{14}C-CO_2 escaped during elution.

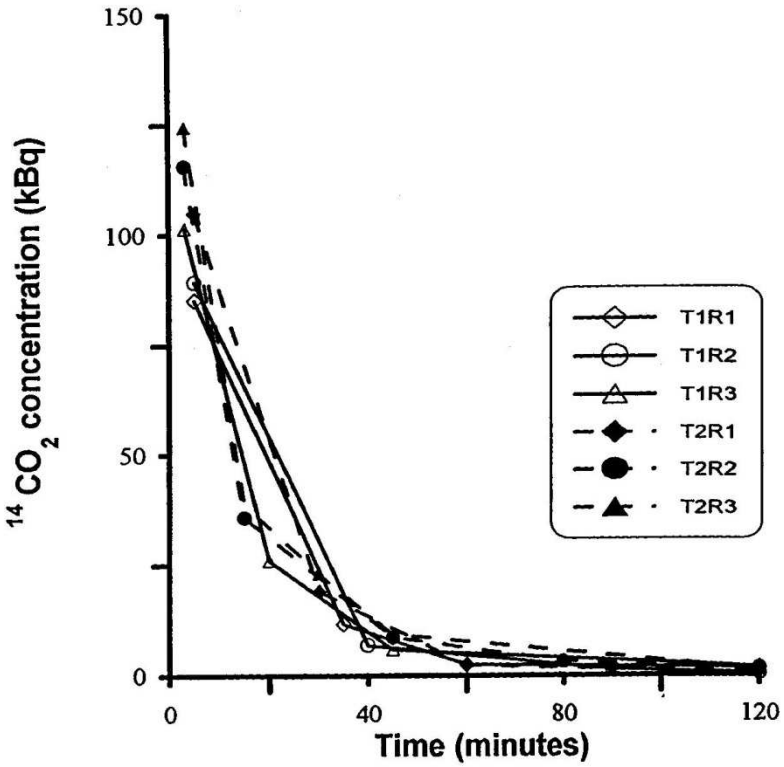

FIG. C.1. ^{14}C-CO_2 concentrations in the chambers at different time intervals after application of the label (T1 and T2 refers to turves maintained at 350 and 700 $\mu L\ L^{-1}$ CO_2 levels, respectively; R1, R2, and R3 the replications).

The results for the soil shoot and root replicate samples gave an average per cent coefficient of variation (% CV) of 0.2 to 11 for the unlabelled C measurements and 5 to 21 for the ^{14}C specific activity (data not shown). Thus, there was good reproducibility between cores for all parameters measured, including specific activity measurements which showed that every even labelling of the whole plant/soil system had been achieved. The level of labelling was sufficient to monitor the specific activity of the components for a lengthy period and showed that the method holds much promise as a field technique

TABLE C.1. DISTRIBUTION OF ^{14}C IN DIFFERENT PASTORAL COMPONENTS, 7 AND 35 DAYS AFTER PULSE LABELLING WITH ^{14}C-CO_2.

Pasture Component	Day	^{14}C (MBq/m^2)	Specific Activity (kBq/g C)	^{14}C in the System (%)
	Above ground			
Shoot	7	106	1504	61
	35	84	517	62
	Below ground			
Whole soil	7	67	17	39
	35	50	10	38
Root biomass	7	25	178	14
	35	28	160	22

The quantities and the proportions of the ^{14}C recovered in herbage, whole soil, and roots, 7 and 35 days after pulse labelling are given in Table C.1. During this period, respiratory ^{14}C-CO_2 lost from the system was not measured. Plausible losses were calculated as a difference between the total amounts of ^{14}C recoveries in the soil-plant system at day 7

minus at day 35. These calculations suggested that 22% of the labelled ^{14}C was lost during this period, presumably by respiration

At day 7 about 61% of the labelled photosynthate was retained within the herbage and 39% translocated below ground into the root biomass (14%), microbial biomass and soil organic matter. During 28 days of pasture growth the herbage production was 204 g dry matter m^{-2} and root biomass 76 g, showing increases of 125% and 20%, respectively. There was no change in the absolute proportions of ^{14}C-labelled photosynthate retained in the herbage (61–62%) and assimilated below ground (38–39%) (Table C.1). However, the specific activity of the herbage declined by a factor of approximately 3, and that of the root biomass by only 10%. There was greater decline in herbage specific activity than was accounted for by the dilution effect of herbage growth. These results indicate that the labelled photosynthate had been either translocated to the below ground component and/or respired. A 20% increase in root biomass during this period and a small change in its specific activity are consistent with the assimilation of translocated labelled photosynthate within the root system.

As mentioned above, under field conditions, about 22% ^{14}C was lost mainly as respiration during day 7 and day 35. Of the remaining ^{14}C, 38% was distributed below-ground in the roots (22%), microbial biomass and soil organic C (16%) (Table C.1). Under closed chambers, 5–7% of the labelled ^{14}C was recovered in the soil 35 days after the application of the label while shoots retained 65–70% and 25–27% was detected in the roots (Table C.2).

TABLE C.2. PER CENT ^{14}C CONTENTS OF SOIL, HERBAGE AND ROOTS SAMPLED AT 4 HOURS, 7 DAYS AND 35 DAYS AFTER PULSE LABELLING THE TURVES MAINTAINED AT 350 AND 700 μL/L CO_2 LEVEL.

	Per cent ^{14}C distribution					
	350 μL/L CO_2			700 μL/L CO_2		
Time	Soil	Herbage	Root	Soil	Herbage	Root
4 hours	1.9±0.3	86.3±1.5	11.9±1.3	2.6±0.2	79.1±1.0	18.3±0.9
7 days	2.1±0.1	78.8±0.3	19.1±0.3	3.4±0.3	75.5±1.5	21.1±1.7
35 days	5.3±0.2	69.8±0.7	25.0±0.5	7.6±0.8	65.2±0.7	27.2±0.4

This study has shown that the simple chamber devised to expose pasture plants to a single pulse of labelled ^{14}C CO_2 in the field, and subsequently monitoring of the ^{14}C in different components was useful in studying C transfers through the plant/soil system.

C.4. Application of the in situ field labelling technique

The *in situ* field labelling technique as described above was used to measure the seasonal and annual C inputs and root decomposition rates of pasture swards in the field [6]. The protocol here is designed to describe the application of the technique under field conditions.

C.4.1. Procedures

C.4.1.1. ^{14}C pulse labelling

In this application study, a representative 25 x 25 m section of a farm was fenced 3–4 weeks prior to ^{14}C pulse labelling of the pastures to exclude grazing animals and for the safe use of the radioisotopes. Six initial labelling times were established by inserting lysimeters at six different seasons of pasture growth, (i.e. spring, late spring, summer, late summer, autumn

and winter). To stimulate the grazed conditions above-ground plants were cut to 20 mm height, one week before ^{14}C pulse labelling. A representative area (250 mm diam.) was pulse-labelled, between 1000 and 1200 h, using the sealed hemispherical chamber made from specially adapted Perspex fishbowl and PVC pipe [7]. The ^{14}C-CO_2 gas was injected into the hemisphere, through a rubber septum. The chambers were removed (after 2 h) and the labelled sward opened up to the environment. Six replicates were labelled at each of the six labelling times.

C.4.1.2. Sampling

Four soil cores (36 mm diam.) were taken to a depth of 100 mm, from each of the six replicates 4 h after labelling (Day 0), and 35 days after labelling (Day 35). The above-ground parts of the labelled pasture plants were clipped from all four cores. Roots were then separated from two soil cores, by gentle shaking and wet sieving. The remaining two cores were sieved (2 mm) for analysis of the soil.

Total C, ^{14}C, total N, pH and cation exchange capacity were determined on air-dry soil. The moisture content of the field-moist and air-dry soil was determined by oven-drying at 105°C to a constant weight. All results are expressed on an oven-dry (105°C) weight basis, unless otherwise stated. Oven-dried (65°C) samples of the above-ground pasture and root biomass were analysed for total C and N.

C.4.1.3 Analyses

Total C in the labelled soils, and plant material was determined following oxidation and digestion using a modified digestion-tube apparatus incorporating a CO_2 traps [4]. A known aliquot of trapping solution was used for liquid scintillation counting and for estimation of total ^{14}C. Total C in the non-labelled soil, and total C in the plant material were analysed by a combustion method (Induction Furnace, Leco, St Joseph, Mich.). A total ^{14}C budget for labelled pools was calculated from the total ^{14}C recovered. The respiratory losses of ^{14}C-CO_2 after 35 days were calculated as the difference between total recoveries of assimilated ^{14}C after these periods and the recoveries after 4 h. Seasonal estimates of the amounts of C assimilated, translocated to roots and added to soil, were made according to [8–9] using the seasonal dry matter production measurements from adjacent areas. It was assumed that, at steady state (Day 35), the distribution of net fixed ^{14}C in the pasture-root-soil system represents the average partitioning of assimilate as:

$$\text{Estimated assimilated C} = (A_{shoot} \times C_{shoot})/(^{14}C_{shoot}) \qquad 1$$

where estimated assimilated C is the seasonal flux (kg C/ha/year); A_{shoot} = seasonal shoot growth (kg C/ha/year); C_{shoot} = shoot C concentration (%); $^{14}C_{shoot}$ = % of net assimilated ^{14}C in shoots at day 35, during that season. The estimated assimilated C during each growth season was then divided among plant-soil components based on the % ^{14}C distribution at Day 35. The sum of seasonal estimates of root and soil C inputs provided annual below-ground C inputs.

To determine the *in situ* root decomposition after clipping the pasture plants in each lysimeter on Day 35, the labelled plant-root-soil system was left for up to one year for the plants to grow under field conditions and initiate root decomposition measurements. Thus the Day 35 after pulse labelling corresponds to Day 0 for the root decomposition. The roots were then sampled periodically at different seasons to determine the amount of total ^{14}C remaining in the roots. The amount of ^{14}C remaining in the roots was plotted against time, assuming a

first-order decomposition rate. The net rate of change of root ^{14}C at any time equals the decomposition rate (V_{dec}), and is proportional to the amount of residual root ^{14}C at that instant. This is expressed by the first order differential equation described in Equation 8 (Paper 3).

The rate constant, k, was estimated by a nonlinear least squares method. The root half-life (time taken by the roots to be reduced to half of the initial value) was calculated as Equation 10 (Paper 3).

C.5. Carbon budget

During the spring (September–October) and autumn (April–May), pasture assimilated (respired plus conserved) the highest amounts of C and the lowest in the late summer (March–April). This implied a decline in C assimilation during the drier February–March period. By using the distribution of ^{14}C after 35 days, the amount of C incorporated into the roots was calculated as 26–39, 10–12, 16 and 16 kg C/ha/d for the spring, summer, autumn and winter seasons. The amounts of C respired and incorporated annually into roots and soil were estimated from the seasonal fluxes. In this regularly fertilised dairy pasture site, 18,220 kg C/ha was respired, 6490 kg remained above-ground in the shoot, and 6820 kg was incorporated into roots, and 1320 kg into the soil.

C.5.1. Root decomposition

Root decomposition rates differed widely with season (Table C.3). Over the first 8 weeks, roots were rapidly decomposed in all the seasons when about 30–50% of the labelled root C was lost

TABLE C.3. DISTRIBUTION OF ^{14}C (PER CENT) IN THE SHOOT, ROOT, AND SOIL OF PASTURE DURING EACH SEASON AT 4 H AND 35 DAYS AFTER PULSE LABELLING. FOR EACH PROPERTY, VALUES FOLLOWED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT AT P ≤0.05; VALUES FOR RESPIRED ^{14}C-CO_2 WERE DETERMINED BY DIFFERENCE AND ARE NOT COMPARED STATISTICALLY[(a)].

System component	Season Spring	Late spring	Summer	Late summer	Autumn	Winter
	4 h					
Shoot	84c	85c	89a	89a	90a	87b
Root	12c	12c	9c	8c	7c	10c
Soil	4.0ab	3.6b	1.2c	3.3b	2.3b	2.5b
	35 days					
Shoot	26de	31d	17f	23e	16f	10g
Root	31a	27a	10c	21b	12c	20b
Soil	4.1ab	5.2a	3.0b	6.7a	5.1a	3.4b
Respired	39	37	70	49	67	66

[a]Calculated as a difference between amounts of ^{14}C-CO_2 in standing herbage, root or soil at 4 hours and 35 days; ND, not determined.

The roots labelled during spring decomposed fastest, with 50% of the label disappearing. Subsequent decomposition was slow, and the rate of root loss followed an

exponential relationship with time. The root decomposition rate constants (k) were 1.7 times higher for the spring-labelled roots than for the autumn-labelled roots. The rate constants for the winter, summer, late summer and late spring roots were similar, and 1.2–1.3 times higher than for autumn roots. The half-life was, therefore, the highest (111 days) for autumn roots and lowest (64 days) for spring roots (Table C.3). The late spring, summer, late summer and winter roots had intermediate half-lives (88–94 days). Only 1–8% of the labelled root C remained at the end of the year.

The results of the study have highlighted the seasonal differences in the assimilation and partitioning of the photo-assimilated ^{14}C, root production and turnover. This study also demonstrated that seasonal changes not only affect the amount of C incorporated into the root biomass (root production), but also affect the output through root decomposition. It is clear from the results that to estimate C budgets accurately for temperate pastures, C turnover models such as Roth-C and CENTURY should account for seasonal changes in below-ground C inputs through root growth and decomposition. The k values as obtained can be used to estimate soil C sequestration.

The sources of this case study are from Reference [6–7].

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D. IN SITU PULSE LABELLING OF PLANTS WITH ^{13}C IN THE FIELD TO EXAMINE PLANT ^{13}C UPTAKE AND ALLOCATION

D.1. Background

Due to handling and safety reasons, increasing use of non-radioactive C isotopes (e.g. ^{13}C) is generally favoured. This isotope can be used in the same manner as the radioactive ^{14}C isotope using the same techniques for labelling plants in the field or growth chamber without the necessity of the handling and safety measures (Paper 4). Thus, ^{13}C labelling is increasingly preferred in isotope labelling studies. The study as described employed an *in situ* ^{13}C pulse labelling of pasture plants in the field similar to that using ^{14}C isotope as described earlier.

The protocol as described here is to present a method for the field in situ labelling of pasture plants with ^{13}C to follow the uptake and incorporation of the ^{13}C into herbage and plant roots. The decomposition of this incorporated ^{13}C is not described in this study. However, the method used for in situ ^{14}C-labelled pasture plants in the field as described in previous paper can be used to measure the decomposition k value substituting ^{14}C with ^{13}C, followed by soil C sequestration determination using Equation 1 (Paper 3).

D.2. Procedures

D.2.1. In situ ^{13}C pulse labelling method

Two long-term research sites in mid-Canterbury, New Zealand were used for ^{13}C labelling. These are the Winchmore and the Tara hill sites in New Zealand. The site at the Winchmore Irrigation Research Station (latitude 43° 47′ south, longitude 171° 48′ east, altitude 160 m asl, 741 mm mean precipitation yr^{-1}), is a flat, border-strip irrigated [1] pasture site on a Lismore stony silt loam soil (Udic Ustochrepts) where fertilizer and irrigation experiments have run since 1952.

The site at the Tara Hills High Country Research Station (latitude 44° 33′ south, longitude 169° 53′ east, altitude 910 m asl, 500 mm mean precipitation yr^{-1}) is a high country, steep (22–33°) oversown tussock species site with an easterly aspect (62–116° north). The soil at the site is a Tengawai gravely silt loam soil (Typic Ustochrepts). The site has been oversown with *T. repens, T. hybridum, T. pratense, L. perenne, Dactylis glomerata, Phleum pratense, F. arundinacea, Agrostis capillaries* and *Cynosurus cristatus* since 1955, and fertilised with both P and S periodically since 1965.

Areas to be labelled were fenced 4–6 weeks prior to labelling to exclude sheep. Plots were labelled within cylindrical steel frames (1.95 m diameter and 0.40 m high) covered with either two layers of 70 μm or one layer of 125 μm thick ethyl-vinyl alcohol film (a multi-layered gas-proof film that is relatively transparent to solar radiation i.e. >90% transmittance, Figure 4.5). The film was sealed to the ground with bags of sand. The air within the canopies was circulated with a vertically mounted electric (12 V, 0.36 A) brush-less fan within a transparent polycarbonate cylinder (0.25 m dia., 0.20 m long). Containers holding aqueous solutions of either $Na_2{}^{13}CO_3$ (99 atom %) or 1.0 g of $Na_2{}^{12}CO_3$ were mounted below the lower edge of the cylinder. At the spring labelling at Winchmore 4.0 g of $Na_2{}^{13}CO_3$ was used whereas 2.5 g was used for all subsequent labelling.

The total CO_2 concentration within the canopy was continuously monitored during labelling with a 'Binos' infra-red gas analyser (IRGA). A trap containing silica gel was connected between the canopy and the IRGA to remove water. After the canopy was sealed and the CO_2 concentration had decreased to near equilibrium, a pulse of $^{13}CO_2$ was released by injecting 3M H_2SO_4 from a syringe through a gas port into the $Na_2{}^{13}CO_3$ solution. After the CO_2 concentration had again decreased to equilibrium, a pulse of $^{12}CO_2$ was released to increase label uptake. The canopy was removed once the CO_2 concentration had again decreased to equilibrium.

D.2.2. Measurements and sampling

Samples of ^{13}C labelled material were collected one hour and 21 days after the end of labelling. At Winchmore, five subsamples per plot were collected and bulked while at Tara Hills, three subsamples were collected and bulked together separately for tussock and inter-tussock samples. Subsamples consisted of herbage material and 25 mm diameter by 200 mm deep soil cores containing both live and dead roots. The samples were temporarily refrigerated. The soil was washed from herbage and root samples using water. All samples were dried at 70°C, the weights of herbage and root samples determined, and were ground (<0.25 mm). Total C and $\delta^{13}C$ were determined on herbage and root samples with a 'Europa Scientific Tracer mass' mass spectrometer. The soil C concentration was measured on composite (12–15 subsamples per sample), with approximately 1 g of air dried, sieved (2 mm) soil being analysed by combustion/infra-red detection of CO_2 using a 'Leco CNS-2000'. The titanium concentration of selected herbage and root samples was measured following nitric/ perchloric acid digestion with an induction coupled plasma technique.

D.2.3. Calculations and statistical analysis

Calculated herbage and root masses were adjusted for soil contamination based on the regression relationships between titanium and C concentrations for each type of sample [2]. Root $\delta^{13}C$ values were adjusted to allow for soil contamination based on the measured root C concentration. The mass of ^{13}C was calculated from the fractional abundance (F) and the total C content using a value of 0.0112372 for R_{PDB} (the absolute isotope ratio of the PDB ^{13}C standard i.e. the ratio $^{13}C/^{12}C$), measured values of $\delta^{13}C$ (‰PDB) and the relationship [3]:

$$F = \frac{{}^{13}C}{{}^{13}C + {}^{12}C} = \frac{\left[\frac{\delta^{13}}{1000} + 1\right] R_{PDB}}{\left[\frac{\delta^{13}C}{1000} + 1\right] R_{PDB} + 1} \qquad (1)$$

The recovery of label ^{13}C was calculated allowing for background ^{13}C and using long-term treatment means of root total C as there was no significant seasonal variation in root mass. Analysis of variance was conducted using the GenStat 5 program for a randomised block design with season within a sub plot stratum for Winchmore and a factorial design using the stocking rate by grazing management interaction as the error term at Tara Hills.

D.3. Results and discussion

D.3.1. ^{13}C recovery and root allocation

The recovery of ^{13}C was greater in herbage than in roots (Figure D.1). The amount of ^{13}C recovered in the herbage decreased between one hour and three weeks, whereas it increased in the roots (except on the irrigation experiment, summer labelled treatments and some inter-tussock treatments (Figure D.1). At 21 days after labelling the ^{13}C root allocation was lower in the superphosphate treatment in Winchmore than in the control, and was lower in spring and summer than in autumn ($p \leq 0.05$ and 0.01 respectively, Table D. 1).

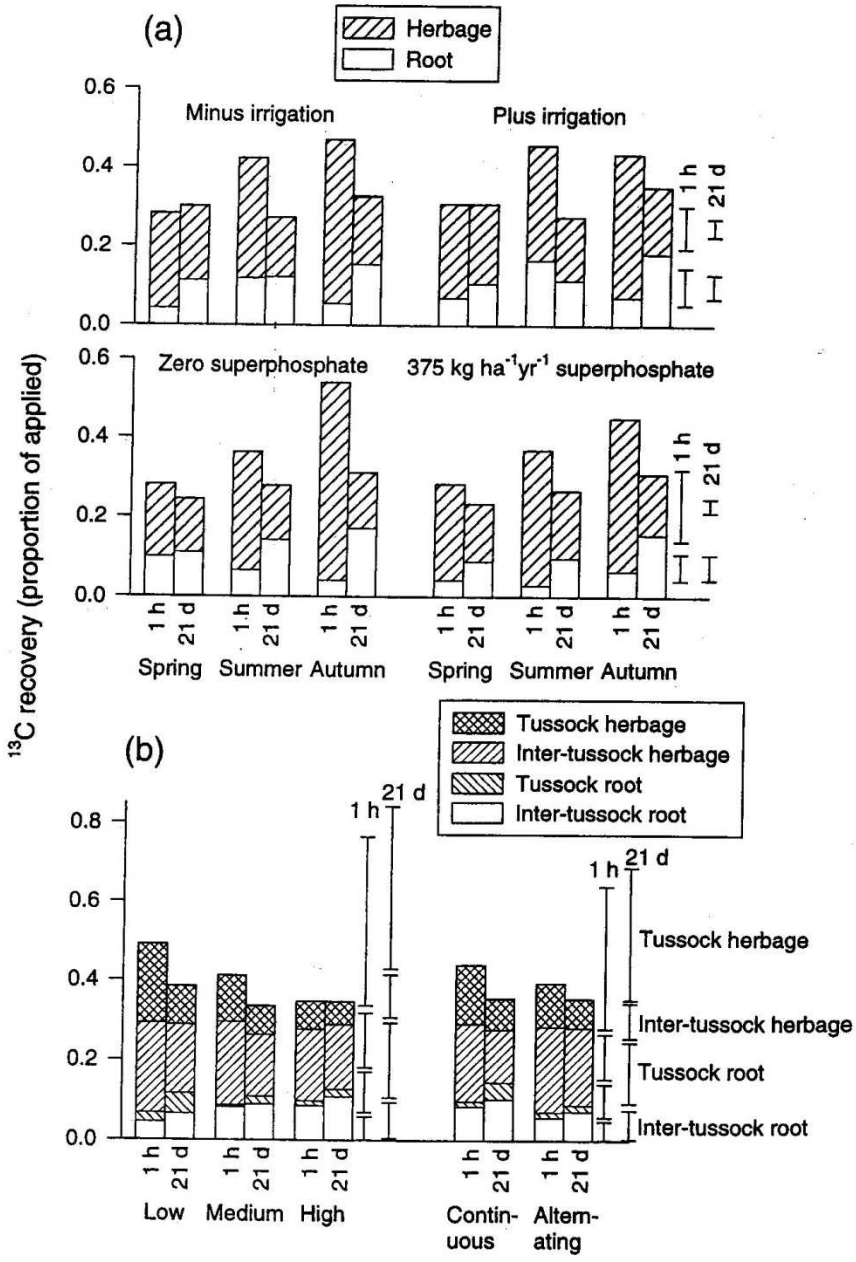

FIG. D.1. Recovery of ^{13}C in pasture herbage and roots, one hour and 21 days after ^{13}C pulse labelling (bars are LSDs, $p \leq 0.05$). (a) Influence of long-term irrigation or superphosphate fertilizer and season of labelling at Winchmore, (b) influence of stocking rate and grazing management at Tara Hills.

At Tara Hills the inter-tussock root C allocation increased with stocking rate at one hour and at 21 days ($p \leq 0.05$, Table D.2).

TABLE D.1. INFLUENCE OF LONG-TERM IRRIGATION OR SUPERPHOSPHATE FERTILIZER AND SEASON OF YEAR WHEN LABELLING ON THE PROPORTION[A] OF PLANT ^{13}C RECOVERED IN ROOTS ONE HOUR AND 21 DAYS AFTER LABELLING AT WINCHMORE

	Irrigation			Superphosphate (kg/ha/yr)		
	0	@ 20%	LSD (P≤0.05)	0	375	LSD (P≤0.05)
Main effect at 1 h	0.18	0.25	0.21	0.20	0.12	0.19
Main effect at 21 d	0.43	0.42	0.11	0.50	0.41	0.08
	Season					
	Spring	Summer	Autumn	LSD (P = 0.05)		
Season main effect 1 h[b]						
Irrigation trial	0.19	0.30	0.15	0.13[c]		
Superphosphate trial	0.24	0.13	0.11			
Season main effect at 21 d[d]	0.39	0.43	0.51	0.05		

[a] *Increase in label ^{13}C mass in roots/(increase in label ^{13}C mass in roots + herbage).*
[b] *Combining treatments within each trial.*
[c] *For trial by season interaction.*
[d] *Combining Winchmore trials.*

TABLE D.2. INFLUENCE OF STOCKING RATE AND GRAZING MANAGEMENT AT TARA HILLS ON THE PROPORTION[A] OF PLANT ^{13}C RECOVERED IN TUSSOCK AND INTER-TUSSOCK ROOTS ONE HOUR AND 21 DAYS AFTER LABELLING

	Stocking rate				Grazing management		
	Low	Medium	High	LSD (P≤0.05)	Continuous	Alternating	LSD (P≤0.05)
Allocation at 1 h:							
Inter-tussock	0.17	0.29	0.33	0.07[b]	0.31	0.21	0.06[b]
Tussock	0.09	0.06	0.12	0.07[b]	0.06	0.12	0.06[b]
Allocation at 21 d:							
Inter-tussock	0.29	0.38	0.40	0.10[b]	0.44	0.27	0.08[b]
Tussock	0.31	0.21	0.34	0.10[b]	0.38	0.19	0.08[b]

[a] *Increase in label ^{13}C mass in roots/(increase in label ^{13}C mass in roots + herbage).*
[b] *Can also be used to compare inter-tussock with tussock.*

This study shows that the ^{13}C pulse labelling method used was successful for field labelling pasture herbage and roots including tussocks. Long-term fertilizer, irrigation and grazing management treatments caused changes in pasture species. Plants showed between and within species adaptations to low soil P by allocating more C to roots and having a larger root mass. However, irrigation had no effect on root C allocation and because herbage production was less in the absence of irrigation the greater root mass of the unirrigated treatment results from a slower rate of root turnover. The translocation of ^{13}C to roots was most rapid in summer. However the greatest ^{13}C enrichment, recovery and allocation to roots by 21 days after labelling were in autumn.

The translocated ^{13}C to the roots can be used to measure the decomposition rate k using the same field study [4] as described earlier for ^{14}C labelled pasture plants (Paper 3) followed by the estimation of soil C sequestration (Equation 1, Paper 3).

The source of this case study is Reference [5].

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E. MEASUREMENT OF SOIL ORGANIC CARBON TURNOVER USING ^{13}C NATURAL ABUNDANCE

E.1. Background

The ^{13}C natural abundance method has been used to determine the MRT or turnover of soil organic carbon [1–3]. This method relies on the difference in ^{13}C natural abundance of C-3 and C-4 plants (Paper 4). It is based on growing C-3 plants on a C-4 soil or vice versa. The rate of C loss from the original vegetation and the incorporation of the new C are inferred from the ^{13}C natural abundance signature of the soil.

The protocol is designed to present the principles of the technique and how it could be adapted to determine soil C sequestration. In addition, an example of an application of the technique to determine ^{13}C natural abundances of C-3 and C-4 plants and their soils is presented.

E.2. Principle of the method

The natural ^{13}C /^{12}C ratios are expressed as δ ^{13}C in Equation 11 (Paper 3). The effect of growing a C-3 plant on a C-4 soil is shown schematically in Fig. 1 where A and B represents different photosynthetic pathway types [3].

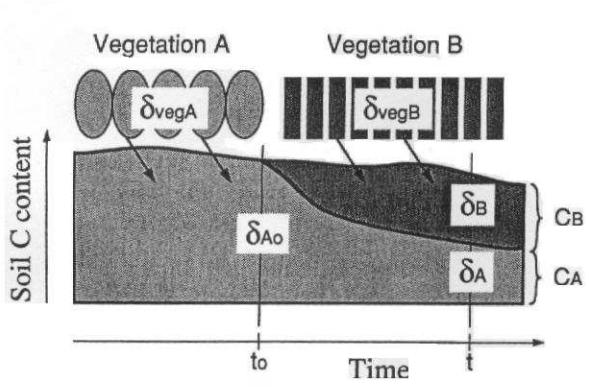


FIG. E.1. Schematic representation of the replacement of coil carbon derived from a vegetation A, by the carbon from a new vegetation B.

At the time of vegetation change (t_o), SOM has an isotopic composition δ_{AO}, which is close to the composition of the original vegetation A. This SOM progressively decays and is partially replaced by SOM derived from the new vegetation B. At a given time *t,* the total C content C is expressed as:

$$C = C_A + C_B \qquad (1)$$

From the mass balance of the heavy isotope, the isotopic composition δ of SOM is given by the following mixing equation; with the approximation that δ unit is related linearly to $^{13}C/C$:

$$\delta(C_A + C_B) = \delta_A C_A + \delta_B C_B \quad (2)$$

where C_A stands for the amount of C derived from the old vegetation A, δ_A for the isotopic composition of that C, C_B for the amount of C derived from the new vegetation B, and δ_B for its isotopic composition. Equation (2) can be rewritten as:

$$F = \frac{C_B}{C} = \frac{\delta - \delta_A}{\delta_B - \delta_A} \quad (3)$$

where F is the fraction of new C in the sample. The function F(t) can be obtained from successive samples taken on the same site or from a chronosequence of sites changed to the new vegetation at different dates. The kinetics of decay of the initial organic C is equivalent to C1 – F), whereas the kinetics of accumulation of the new C is equivalent to CF. Under steady state, F is the direct expression of the turnover of soil C. As δ of the soil sample is related linearly to F, its change with time gives a rather direct expression of turnover of soil C.

The F value is the k value given in Equation 13 (Paper 3) and the MRT is calculated from Equation 12 (Paper 3). From this k value, soil C sequestration can be estimated from Equation 1(Paper 3). The soil C sequestration is not determined in the studies of [3, 5].

Equation (3) represents the strict mixing equation relating to F to δ, but the values of δ_A and δ_B cannot be measured directly and must be estimated. In fact, there is evidence that the isotopic composition of SOM is slightly different from that of the plant material from which it derives. However, most investigators using the natural ^{13}C-labeling technique assume δ_B is equivalent to the isotopic composition of the new vegetation B or its litter (i.e., δ_{vegB}), and δ_A is equivalent to the initial $\delta^{13}C$ of the soil or, more frequently, to the isotopic composition of soil at a reference site kept under the initial vegetation (i.e. δ_{refA}). Under these assumptions, Equation 3 then becomes:

$$F = \frac{\delta - \delta_{refA}}{\delta_{vegfB} - \delta_{refA}} \quad (4)$$

At a given site, the average $\delta^{13}C$ difference between C_4 and C_3 vegetation is approximately 15‰. This difference is the maximum magnitude of the tracer. The small isotope effects or fractionations that occur in soils could be taken as a noise.

A third equation (Equation 5) which is slightly different from Equation 4 to be applied also to the analysis of the changed site, compared to a reference with the vegetation unchanged was proposed by (Balesdent and Marriotti, 1996) as

$$F = \frac{\delta - \delta_{refA}}{\Delta} \quad (5)$$

where δ is the isotopic composition of the soil sample and δ_{refA} is that of the corresponding soil sample taken from a reference site kept under the initial vegetation, as in Equation 5. But Δ is equal to the average difference between the $\delta^{13}C$ of the vegetation in the studied site and in the reference site, over the duration following the vegetation change ($\Delta = \delta_{vegB} - \delta_{vegA}$).

This equation will be unbiased, provided that both sites have a similar history and similar C dynamics. It assumes that, at date t_0, the isotopic composition of the vegetation was increased or decreased by a constant value (Δ). Its advantage relative to Equation 4 is that it can be applied even if the isotope enrichments during C decay are high. It can even be applied

to SOM fractions with an isotopic composition very different than that of the vegetation itself. It includes the historical (last century) change of the atmospheric CO_2- $\delta^{13}C$, provided that C_4 and C_3 plants were affected by this change in the same manner. It can also be applied to cases where multiple vegetation changes may have occurred before the change actually chosen for study, as in the case of C_4 tropical ecosystems, where old forest organic matter is often found in significant quantities in deep horizons [6]. In addition, this equation can also be applied to sites initially under mixed C_3-C_4 vegetation, or to sites with a complex isotopic history, that were then converted to two types of use: one with a C_4 vegetation and one with a C_3. This equation (Equation 5) will be preferred to Equation 4 in systems where C dynamics are close to steady state.

E.3. An example of the application of the ^{13}C natural abundance method to measure long- and medium-term turnover of soil organic matter

E3.1. Procedures

The study was based on the variations of ^{13}C natural abundance induced by the repeated growing of maize *(Zea mays*) with a high $\delta^{13}C$ (C-4 photosynthetic pathway) value on a soil which has never carried any such plant. The δ^{13} value being about equal to that of the plant materials from which it is derived thus changing (by the ^{13}C content of the initial organic C inputs ($\delta^{13} <= -26‰$) to that of the maize plants ($\delta^{13}C = -12‰$).

The soil samples studied were obtained from two experimental fields of the Institut National de la Recherché Agronomique. Both are situated in south-western France.

In Auzeville (Haute-Garonne), the sampled soil is a silt clay on an alluvial deposit (Eutrochrept). Three plots, P1 to P3, which have been cropped continuously with corn for 13 years, without any organic manure, have been sampled every two years since the beginning of the experiment. After harvest, leaves and stalks were incorporated back into the soil. In Doazit (Landes), the sampled soil is a weakly weathered sandy loam on an eolian deposit (Hapludalf). The experiment plots have been continuous corn cultivation for 23 years, after pine-forest clearing. In a first trial, T1, leaves and stalks were incorporated back into the soil; in a second trial, T2, they have been removed for the last 17 years. Equivalent samples in an adjacent plot under forest vegetation (*Pinus, Quercus* and *Pteridae*) were also collected. Average yields of grain were 7.5 t ha^{-1}. No organic manure was applied.

Representative composite soil samples were obtained from 12 cores of 0.4 kg soil collected in each plot, mixed together, air-dried and crushed (<2 mm). Samples from Auzeville were then gently ground through a 200 μm sieve, in order to remove coarser plant fragments. Samples from Doazit were separated into particle size fractions. Soil was firs disaggregated by mechanical shaking in water, with 5 mm dia glass balls. Sand and sand-sized plant fragments were separated at 200 and 50 μm by wet sieving. Further dispersion of the resulting 0–50μm suspension was obtained by stirring with sodium metaphosphate (1 g L^{-1}).

The fine clay fraction (0–0.2 μm) and coarse clay fraction (0.2–2 μm) were separated by centrifugation and freeze-dried. The silt fractions (2–20 and 20–50 μm) were separated by sedimentation and oven-dried at 40°C. Each separation was obtained by five successive sedimentations. Fine clays were flocculated by addition of 10 g L^{-1} NaCl; water soluble material was discarded. All fractions were then ground by hand in a mortar.

Total C and N were determined with a CHN autoanalyser (Carlo Erba 1106). Carbon isotope ratios of plant and soil organic samples were measured on the CO_2 obtained by

combustion under pure oxygen atmosphere at 900°C. The gas was then purified and analyzed on a mass spectrometer fitted with triple ion collectors and dual inlet system equipped for rapid switching between reference and sample (VG SIRA 9 and Finnigan Delta E). The laboratory reference has been calibrated *vs* PDB, using the international standard now available, NBS 19.

E.4. Results and discussion

In Auzeville, the $\delta^{13}C$ value of the initial organic matter of soil sampled from the meadow plots (C-3 vegetation), considered as reference (initial δ_0 value), was found to be –26.2‰. In Doazit, $\delta^{13}C$ of bulk forest topsoil was –26.7‰.

In both fields studied, the mean value of $\delta^{13}C$ for maize leaves and stalks was –12.4‰. Roots had $\delta^{13}C$ values of –12.2‰ in both cases. At Auzeville after 13 years of continuous corn cultivation, $\delta^{13}C$ rose from δ_0 = 26.2 to –23‰. These values mean that x = 22% (x = percent C derived from C-4 plant) had turned-over since the beginning of the experiment. The x value is obtained from the following equation as

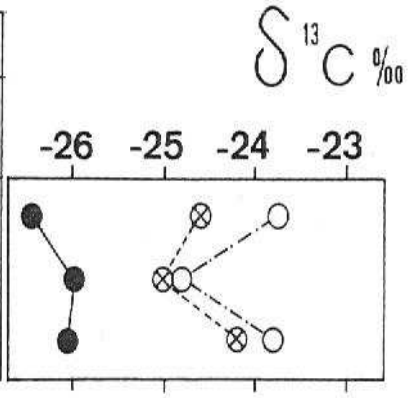
$$A = \frac{x}{100}.A_1 + (1 - \frac{x}{100}).A_0 \qquad (6)$$

where $A = {}^{13}C/({}^{12}C + {}^{13}C)$ after a time t of cultivation at time t, $A_0 = {}^{13}C\,({}^{12}C + {}^{13}C)$ for the initial soil at t = o.

For a similar soil under C-3 vegetation, $A_1 = {}^{13}C/({}^{12}C + {}^{13}C)$ for the C-4 plant. The total organic C at 13 years is 8.5 mg/g. Of this, 78% is C from the original soil, that is 6.6 mg/g in 13 years. Assuming exponential decay, the turnover time is 13/ln(6.6/9.5) = 36 years.

For the Doazit soil, two treatments (T1 and T2) were compared. As shown in Fig. E.2., within the topsoil (0–30 cm) of T1 treatment, 19% of the C had turned over after the 23 years of experimentation, corresponding to an absolute quantity of 2.8 mg/g of the 0–50 µm fraction. This quantity, which corresponds to about 650 g/m^2, is less than the one calculated for Auzeville soil, where 750 g/m^2 came from corn after only 13 years of cultivation. This difference can be explained by the low clay content and the slight acidity of Doazit's soil. For the T2 treatment, the quantity of C from corn in the fraction 0–50 µm was only 1.7 mg/g fraction. These inputs to the soil represent only 60% of the input in treatment T1 resulting in a higher total organic C content in the T1 treatment.

Depth (m)	T1 trial Organic C mg.g^{-1}	x %	T2 trial Organic C mg.g^{-1}	x %
0-30	14.5	19	12.7	13
30-40	7.0	8	5.4	7
40-60	3.5	17	4.6	13



x = percent of organic carbon from corn

● forest soil

○ T1 : restitution of leaves and tips

⊗ T2 : no restitution

FIG.E. 2. Organic carbon content and $\delta^{13}C$ of 0–50 μm fractions in a soil under forest (which acts as a reference) and under soils after 23 years of continuous corn cultivation (Doazit experimental plots T1 and T2).

This study shows that the cultivation of a C-4 plant on soils which had never carried any such plant appears to provide a useful ^{13}C labelling of SOM. It can be applied to the study of bulk SOM dynamics and turnover and to the characterization of organic fractions. With a $\delta^{13}C$ precision of about 0.3‰, it gives an absolute precision of 2% on the proportion of 'labelled' plant material incorporated to the soil, from 0 (δ = –27‰) to 100% (δ = –12‰). Thus, this study illustrated the use of ^{13}C natural abundance to estimate the turnover or decomposition value k, which can then be used to estimate soil C sequestration.

The sources of this case study are Reference [3, 5].

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F. ESTIMATING THE TURNOVER OF SOIL ORGANIC MATTER USING RADIOCARBON DATING

F.1. Background

^{14}C dating data have been used to estimate turnover rates of SOM fractions (e.g. [1–5]. The turnover time τ is estimated and from this the decomposition rate k is estimated as $^1/_\tau$. With the k value, soil C sequestration can be estimated using Equation 1 (Paper 3). The turnover time, τ, represents the time C resides in the soil and plant system and may underestimate the actual decomposition rate, k, of the C resides for several years in the living roots, plant or woody material in trees [6].

The protocol presented here is to show how the turnover time (τ value) of SOM fractions can be determined using radiocarbon dating data.

F.2. Principle of the method

The radiocarbon dating method can be used to estimate soil C turnover time and turnover rates (e.g. [2–3, 7–8]

The radiocarbon activity Δ of a soil organic carbon sample is related to its age density distribution f(t) [1] by:

$$\Delta = \int_{\circ}^{\infty} f(t)\, \Delta_o\, (p-t)\, \exp(-k_1 t)\, dt \qquad (1)$$

where f(t) is the age density distribution (= fraction of total carbon, with age t),

$$\int_{\circ}^{\infty} t f(t)\, dt = 1$$

$\Delta_o(x)$ is the ^{14}C activity of atmospheric CO_2 at year X.

p is the date of sampling.

k_1 is the radiocarbon decay constant, $k_1 = 1{,}210\ 10^{-4}$ year^{-1}.

If one assumption is made on the age density distribution, then dynamic parameters can be calculated by solving Equation 1 (e.g. mean age $\alpha = \int_{\circ}^{\infty} t\, f(t)\, dt$). In the case of a steady state (e.g. as an assumption), the turnover time is given by $\tau = 1/f(o)$. The decomposition rate k is given by $k = {}^1/_\tau$ (Equation 19, Paper 3).

F.3. Procedure

The study by [1] is presented here as an example of the use of the radiocarbon dating method to estimate the turnover time (τ). Two meadow topsoil (2–12 cm) were sampled from the Jura mountains, in eastern France (T = 5°C, P = 1200 mm). Soil A is a Rendoll. Soil B is an Hapludalf. A SOM fractionation scheme was used as described below. Coarse plant debris were removed by wet sieving at 100 μm after dispersion in water with glass balls. Fraction 0–100 μm was divided and treated:

(i) One hand by successive alkaline extractions with NaOH adjusted at pH 10 (Extract I) then 0.1M $Na_4P_2O_7$, pH 9.8 (Extract II). Fulvic acid (FA) fractions were separated from humic acid (HA) at pH 1.5. Within humin, particle-size fractions 0–0.2 μm and 0.2–2 μm were separated and hydrolysed with boiling 3M HCl.

(ii) On the other by a step-by-step hydrochloric acid hydrolysis. The first hour and 2–20 hours hydrolysates were separately neutralized at pH 5.5. A soluble fraction was so separated from a co-flocculated fraction (non sol.).

The ^{14}C was measured by a liquid scintillation counting during 24 to 72 hours in pure benzene obtained from the sample C.

$\Delta^{14}C = (A_S / A_R - 1) \times 1000$, where A_S is sample activity, A_R is reference activity, Δ unit is corrected for isotope effect.

F.4. Results and discussion

The ^{14}C content of the fractions is shown in Fig. F.1. Mean ages are calculated assuming an exponential age density distribution, in which case turnover time and mean age have the same value.

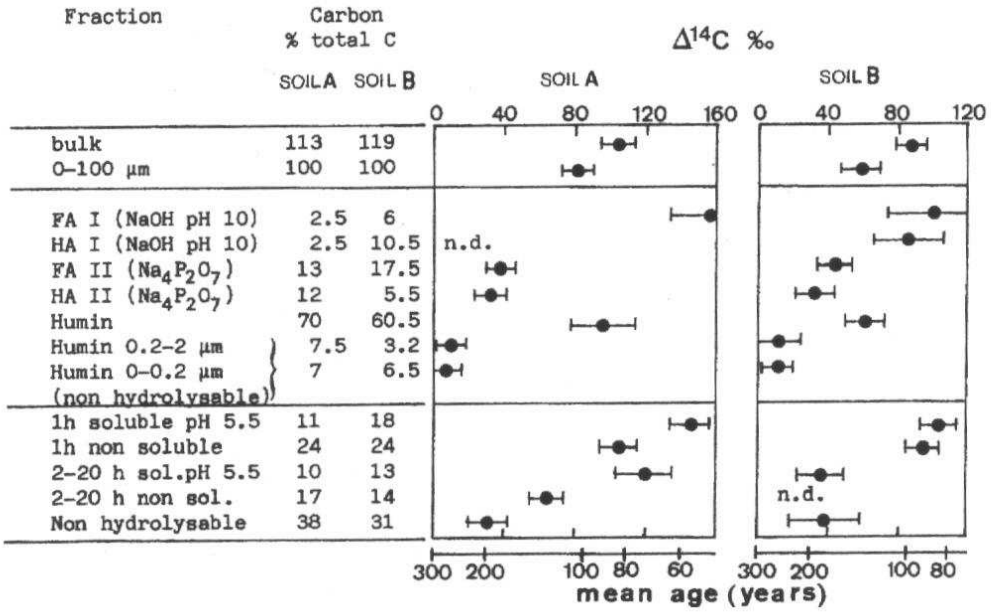

FIG. F.1. Results showing ^{14}C content and mean age of soil organic fractions [1].

Plant debris >100 μm was the youngest fraction (<15 years). Organic matter extracted by the complexing power of $Na_4P_2O_7$ (Extract II) was older than organic matter extracted at the same pH by NaOH (Extract I). This shows the role of the amorphous mineral phase, mainly Fe oxides in these soils, on the stabilization of SOM. In each extract, HA and FA showed the same age. A mean age value was obtained for humin, but the fraction was shown to be heterogeneous. A non-hydrolysable humin material associated with either fine clay or coarse clay particle size fraction was the oldest separated in this study (280 years), but it represented less than 15% of the total carbon.

The first hour acid hydrolysate, which was soluble at pH 5.5 contained 70% of the soil sugars, and was the youngest fraction (60–80 years) showing an age similar to that of FA I. Non-hydrolysable material was found to be the oldest fraction (200–170 years) and this is consistent with other reports[10–11] .

The results are found to be generally similar for the two soils, even though each represented a soil type distinctly different from the other. This study showed that the turnover time τ can be determined. It can then be used to calculate the decomposition rate k ($k = {}^{1}/_{\tau}$) and then soil C sequestration according to Equation 1 (Paper 3).

The source of this case study is Reference [1].

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ABBREVIATIONS AND ACRONYMS

AR	Artificial radiocarbon
FAO	Food and Agriculture Organization of the United Nations, Rome
FANci	Fisher ANalysen Instrumente
GHGs	Greenhouse gases
ILO	International Labour Organization
IMO	International Maritime Organization
IPCC	Intergovernmental Panel on Climate Change
iPOM	Intra-aggregate particulate organic matter
LSC	Liquid scintillation counter
MRTs	Main residence times
NT	No tillage
OECD/NEA	Organisation for Economic Co-operation and Development / Nuclear Energy Agency
PAHO	Pan American Health Organization
SOC	Soil organic carbon
SOM	Soil organic matter
UNEP	United Nations Environment Programme
UNFCC	United Nations Framework Convention on Climate Change
WHO	World Health Organization

CONTRIBUTORS TO DRATING AND REVIEW

Adu -Gyamfi, J.	Soil and Water Management and Crop Nutrition Section, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, Vienna, Austria
Goh, K. M*.	Soil and Physical Science Group, Agriculture and Life Sciences Division, Lincoln University Canterbury, New Zealand
Nguyen, M-L.	Group One Consultancy Limited Optimizing Nutrient Efficiency St Heliers, Auckland 1740
Zapata, F.	Consultant, Soil and Water Management and Crop Nutrition Section, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, Vienna, Austria

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