Published 1994

Chapter 39

Isotopic Methods for the Study of Soil Organic Matter Dynamics

DUANE C. WOLF, *University of Arkansas, Fayetteville, Arkansas*

J. O. LEGG, *University of Arkansas, Fayetteville, Arkansas*

THOMAS W. BOUTTON, *Texas A & M University, College Station, Texas*

The vast majority of all organic C and N in the world's terrestrial environment is present in the form of soil organic matter, which contains approximately 1.5×10^{18}g of C (Post et al., 1982) and 0.095×10^{18}g of N (Post et al., 1985). In addition to its importance as a reservoir of C, N, and other nutrients, this pool of soil organic matter has many properties that define the structural and functional attributes of natural and agricultural ecosystems.

The benefits of soil organic matter in crop production have been recognized for centuries (Allison, 1973), although the reasons for the beneficial effects have not been clearly understood. Organic matter is highly important in relation to soil aggregation, tilth, cation exchange capacity, nutrient supply, soil water, biological activity, and other soil characteristics. Repeated additions of organic matter to soils, normally occurring from plant and animal residues, and subsequent biological, chemical, and physical processes that occur, create a highly complex and dynamic system. This complex system attracted the attention of chemists as early as the 18th century when acid and alkali extraction procedures were first used to remove organic matter from soils (Russell, 1961). In the 1940s, isotopes of C and N came into use in studies of transformations of these elements during organic matter decomposition. Paul and van Veen (1978) reviewed these early studies and proposed a model to describe the rate of organic matter decomposition. It is important to emphasize that, in the decomposition of labeled compounds, other organic compounds may be synthesized simultaneously from the mineralized C and N. The position of the labeled atom

 Methods of Soil Analysis, Part 2. Microbiological and Biochemical Properties—SSSA Book Series, no. 5.

in the material undergoing mineralization can influence the results from decomposition studies.

Soil organic matter not only has fundamental importance at the ecosystem level, but also has considerable importance for global biogeochemistry due to the size of the pool and its linkages to atmospheric CO_2 via primary production and decomposition. There is currently much interest and speculation regarding whether soil organic matter is a net carbon sink or source in the global carbon cycle under present environmental conditions as well as under conditions of elevated atmospheric CO_2 concentration and altered climate (Prentice & Fung, 1990; Schlesinger, 1990). As a result of the controversial role of soil organic matter in global biogeochemistry, methodologies for quantifying fluxes of soil organic matter have become extremely important.

In this chapter, we will address isotopic methods of assessing fluxes associated with soil organic matter. There are basically four approaches available: (i) use of organic matter labeled with ^{14}C or ^{13}C; (ii) use of organic matter labeled with ^{15}N; (iii) use of natural variation in ^{13}C in organic matter; and (iv) use of ^{14}C injected into the atmosphere during nuclear weapons testing. Approaches 1 and 2 are most useful for short-term studies of 1 to 10 yr, and approaches 3 and 4 are useful for examining fluxes on time scales ranging from tens to thousands of years. Since methods involving the use of "bomb" ^{14}C have been reviewed recently (Harrison et al., 1990; Goh, 1991), we will address some of the more common methods of using ^{13}C, ^{14}C, and ^{15}N to study organic matter decomposition. The References section will provide citations for obtaining further information on pertinent isotopic methodology as well as related analytical techniques.

39–1 DECOMPOSITION OF ^{14}C-LABELED ORGANIC MATTER IN SOILS

39–1.1 Introduction

Historically, the use of ^{14}C-labeled materials to study soil organic matter decomposition has been accomplished by adding radiolabeled plant material, microorganisms, microbial products, or specific compounds to the soil and measuring the amount of $^{14}CO_2$ evolved during an aerobic incubation (Ladd & Martin, 1984; Wolf & Legg, 1984). Various methods are available to produce the radiolabeled materials and to collect the evolved $^{14}CO_2$.

The objective of this section is to present methods for the production of ^{14}C-labeled materials and define several methods available for the collection and assay of the $^{14}CO_2$ evolved during aerobic laboratory incubation studies. It is not our intent to define all available techniques, but rather to provide details of the more commonly used procedures. Using even a weak beta-emitting radioactive isotope such as ^{14}C requires particular at-

tention to safety considerations. The use of radioisotopes generally requires a permit or license and training in radiation safety. The aspects of health and safety will not be addressed in this section, but any researchers undertaking a project involving ^{14}C should contact their local Radiation Safety Officer for details on licensing and permits.

39–1.2 Obtaining ^{14}C-Labeled Organic Materials

Obtaining ^{14}C-labeled materials for studies on organic matter decomposition is the first and possibly the most difficult step. The choice of organic material may be dictated by the objective of the given research project and the availability of specific research facilities in which to carry out labeling experiments. Products that can be labeled range from the simplest of pure compounds purchased from several commercial sources (Table 39–1) to whole plants grown in a $^{14}CO_2$ environment or microorganisms and microbial products produced in the laboratory on a wide range of labeled organic substrates. Obviously the cost, time, and facilities will have an impact on the organic material available for any given experiment.

39–1.2.1 Labeling Plant Material

One method used to produce ^{14}C-labeled plant material is to grow plants in a $^{14}CO_2$ environment in a growth chamber and harvest the plants after a suitable growth period. Another approach is to inject a ^{14}C-labeled precursor directly into the plant and let the metabolic activity of the plant incorporate the label into various biochemical fractions. Both methods require an appropriate containment facility to prevent contamination of the laboratory environment.

39–1.2.1.1 Materials

1. $^{14}CO_2$ or ^{14}C-labeled precursor.
2. Suitable growth and containment facility.

39–1.2.1.2 Procedure. Details of construction and operation of $^{14}CO_2$ growth chambers have been presented by several researchers (Andersen et al., 1961; Cheshire & Griffiths, 1989; Harris & Paul, 1991; Jenkinson, 1960; Scully et al., 1956; Smith et al., 1962; Warembourg & Kummerow, 1991).

At harvest, the plant may be separated as desired into various components such as shoots, leaves, and roots. Once the plant material is harvested, it should be lyophilized. Oven drying the plant material has the potential to release $^{14}CO_2$ into the laboratory environment and is not recommended. After freeze-drying, the material can be ground and sieved to the required size.

A second method of labeling plant material is to treat growing plants with ^{14}C-labeled precursors of lignin or cellulose biosynthesis. Haider et al. (1977) used a syringe to inject ^{14}C-labeled *p*-coumaric acid, a precursor in the synthesis of lignin, into the base of young corn (*Zea mays* L.)

Table 39–1. Partial listing of commercial sources of ^{14}C-labeled materials or liquid scintillation counting cocktails.

Company name and address	Telephone number	^{14}C-Labeled material	LSC cocktail
American Radiolabeled Chemicals, Inc. 11624 Bowling Green Dr. St. Louis, MO 63146	1-800-331-6661	X	
Amersham Corp. 2636 S. Clearbrook Dr. Arlington Heights, IL 60005	1-800-323-9750	X	
Curtin Matheson Scientific, Inc. P.O. Box 1416 Houston, TX 77251-1416	1-800-879-2670	X	X
Dupont Co., Biotechnology Systems 549 Albany St. Boston, MA 02118	1-800-551-2121	X	X
Fisher Scientific 900 Stewart Ave. Plano, TX 75074	1-800-766-7000		X
ICN Biomedicals, Inc. 3300 Hyland Ave. Costa Mesa, CA 92626	1-800-854-0530	X	X
Isolab, Inc. Drawer 4350 Akron, OH 44321	1-800-321-9632		X
Isotope Products Laboratories 1800 N. Keystone St. Burbank, CA 91504	1-818-843-7000	X	
Moravek Biochemicals, Inc. 577 Mercury Lane Brea, CA 92621	1-800-447-0100	X	X
Packard Instrument Co. 800 Research Parkway Meriden, CT 06450	1-203-238-2351	X	X
Research Products International Corp. 410 N. Business Center Dr. Mount Prospect, IL 60056	1-800-323-9814		X
Sigma Chemical Co. 3050 Spruce St. St. Louis, MO 63103	1-800-325-3010	X	X

plants. The plants were injected three times and allowed to grow an additional 3 wk before harvest. The plant tops were harvested and small molecular weight compounds extracted before the material was used for decomposition studies. Similarly, lignin components of white oak (*Quercus albus*), red maple (*Acer rubrum*), and cattail (*Typha latifola*) have been labeled by feeding plants aqueous solutions of L-[U-^{14}C]phenylalanine or [2′-^{14}C(side chain)] ferulic acid through their cut stems (Crawford, 1978; Crawford & Crawford, 1976). Cellulosic components of lignocelluloses were labeled by substituting solutions of D-[U-^{14}C]glucose (Crawford et al., 1977).

39–1.2.1.3 Comments. Safety is critical when using radioisotopes, and it is important to prevent contamination of the laboratory (Coleman & Corbin, 1991). Always wear a laboratory coat, chemical safety goggles, and disposable gloves. Work on absorbent paper and use a pipette bulb for all pipetting. Avoid being exposed to ^{14}C-labeled aerosols, dust, and volatile material. All grinding and sieving procedures should be conducted in a properly vented fume hood. It is obvious that there should be no eating, drinking, or smoking in the laboratory at any time. The growth medium may be contaminated with ^{14}C, and suitable disposal procedures must be followed.

39–1.2.2 Labeling Microbial Biomass and Microbial Products

Most studies using ^{14}C-labeled microbial biomass involve incubation of a pure culture of a given microorganism with a suitable labeled substrate such as uniformly ^{14}C-labeled glucose. The microbial biomass is harvested and lyophilized following an appropriate incubation period. The dry biomass can be ground and sieved before it is added to the soil in an incubation experiment.

39–1.2.2.1 Materials

1. ^{14}C-labeled substrate such as D-[U-^{14}C]glucose.
2. Pure culture of microorganism (see American Type Culture Collection Catalog).
3. 250-mL Erlenmeyer flasks.
4. Orbital shaker.
5. System to collect evolved $^{14}CO_2$ (see Fig. 39–1, 39–2, 38–2).
6. Freeze dryer.
7. Autoclave.

39–1.2.2.2 Procedure. Prepare a growth medium suitable for the microorganism of interest. Add an appropriate amount of ^{14}C-labeled substrate to the medium, add the inoculant, and connect the flask to the CO_2 collection system (see Fig. 39–1 and chapter 38 in this book). Place the 250-mL flask on the shaker and incubate until sufficient microbial growth has occurred. The microbial biomass can be harvested by centrifugation and then lyophilized, ground, and sieved before it is used in soil decomposition studies. Wagner and Krzywicka (1975) used $^{14}CO_2$ to label algal biomass and D-[U-^{14}C]glucose was used by Reyes and Tiedje (1973) to produce labeled *Saccharomyces cerevisiae*. Labeled whole cells have been fractionated and their components used in soil decomposition studies (Hurst & Wagner, 1969; Nakas & Klein, 1979). Measurement and turnover of microbial biomass have been evaluated (Jenkinson & Powlson, 1976; Kassim et al., 1981, 1982) and reviewed (Jenkinson & Ladd, 1981; Wagner, 1975).

Additionally, ^{14}C-labeled microbial products such as polysaccharides, polyphenols, and proteins have been isolated and used in decomposition studies. Oades and Wagner (1971) and Zunino et al. (1982) used a mineral

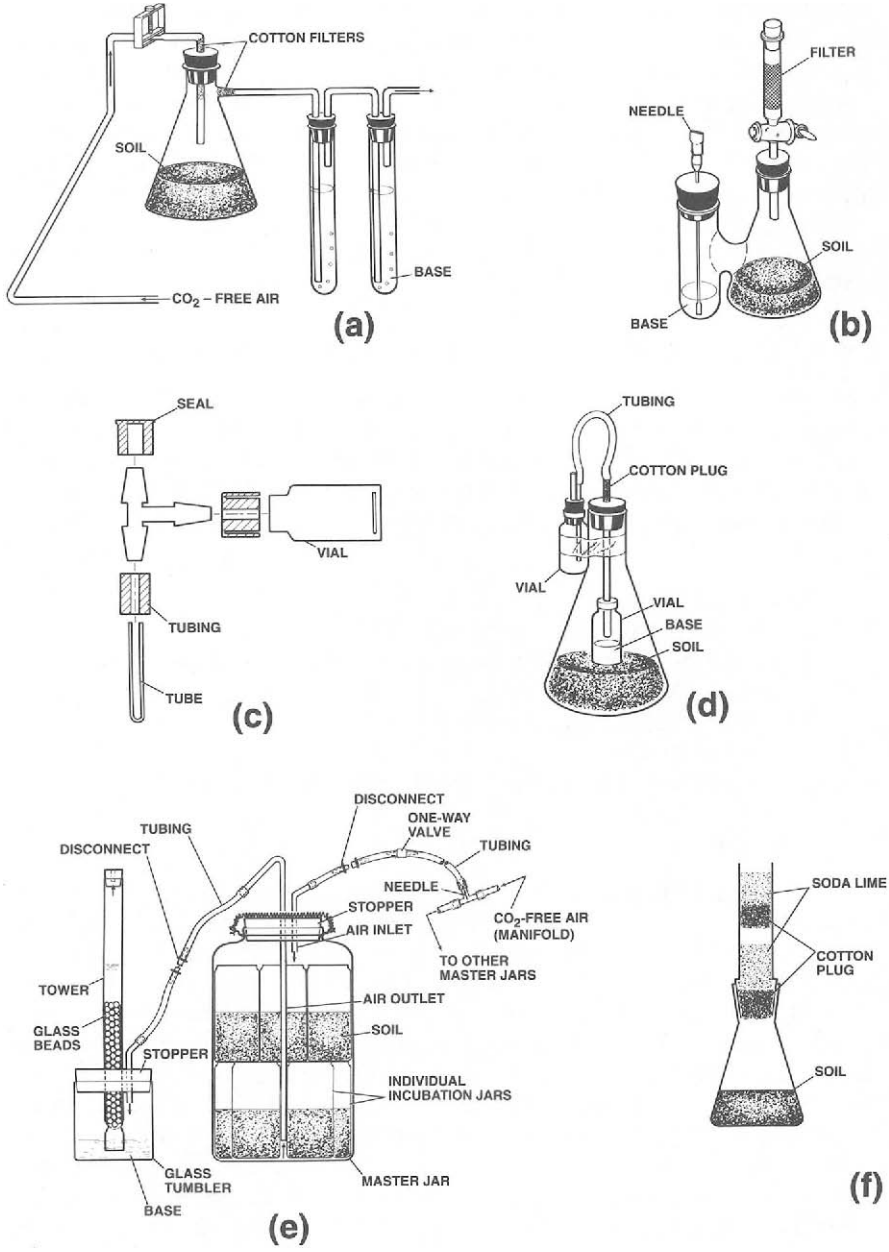

Fig. 39–1. Common units to collect $^{14}CO_2$: (a) flow-through system (Atlas & Bartha, 1972); (b) biometer flask (Bartha & Pramer, 1965); (c) tube with attached scintillation vial (Sissons, 1976); (d) static flask unit (Loos et al., 1980); (e) flow-through system (Stotzky et al., 1993); (f) static flask chamber (Anderson, 1982).

salts medium supplemented with D-[U-^{14}C]glucose to grow *Leuconostoc* spp. The polysaccharide material was collected and used in decomposition studies. Soil fungi have been grown on a mineral salts medium with D-[U-^{14}C]glucose to produce melanins used in laboratory incubations (Martin & Haider, 1979; Martin et al., 1972, 1982). The ^{14}C-labeled proteins produced by *Chlorella pyrenoidosa* and *Microcoleus* spp. also have been obtained (Verma et al., 1975).

39–1.2.2.3 Comments. The spent microbial growth medium containing ^{14}C-labeled material must be disposed of in a suitable manner. It is important to realize that a radioactive waste container with spent medium can be a source of $^{14}CO_2$ in the laboratory if microbial growth occurs.

39–1.2.3 Specific ^{14}C-Labeled Compounds

In certain studies related to organic matter decomposition, it is desirable to amend the soil with specific compounds such as an amino acid, phenol, or sugar (Haider & Martin, 1975). These compounds may be labeled in a specific position in the molecule, or they may be labeled uniformly. In most cases, the compound can be obtained from commercial sources, a few of which are listed in Table 39–1. Some companies also have a service available to synthesize special compounds.

39–1.3 Methods and Approach to Incubations of ^{14}C-Labeled Organic Materials

It is not possible to present all of the methods that have been used to determine decomposition of ^{14}C-labeled organic materials in soil. References should be consulted for additional methods or specific details of more specialized incubation systems (Anderson, 1982; Marvel et al., 1978; Stotzky, 1965). Some of the common systems are presented in Fig. 39–1. In general terms, the soil is placed in a flask and amended with the ^{14}C-labeled organic material and attached to a CO_2 collection unit (see chapter 38 in this book). For aerobic incubation studies, O_2 must not be a limiting factor. For this reason, a continuous flow-through system has been used. The evolved $^{14}CO_2$ can be trapped in a base such as KOH or NaOH and the ^{14}C activity assayed by liquid scintillation counting techniques. Several static systems have also been used and have the advantages of a simple design and conservation of space.

39–1.3.1 Materials

1. Liquid scintillation spectrometer.
2. Liquid scintillation counting (LSC) cocktail.
3. Scintillation vials.
4. Pipettes.
5. KOH or NaOH containing 10 mg/L Tropaeolin O (Aldrich Chemical Co.).

6. Soil, field moist and sieved.
7. ^{14}C-labeled organic material.
8. $^{14}CO_2$ collection unit (see Fig. 39–1, 39–2, and 38–2).

39–1.3.2 Procedures

The amount of ^{14}C activity to add to the soil will depend upon the amount of ^{14}C expected to be found in the fraction of interest or evolved as $^{14}CO_2$, analytical efficiency of the laboratory procedure that determines whether the activity in the fraction of interest is diluted or concentrated, and the minimal activity in the LSC cocktail to give efficient counting (Voroney et al., 1991). It is generally necessary to determine the counting efficiency for each procedure and LSC cocktail used and it may be necessary to do a preliminary or pilot study to determine the appropriate levels of activity to add. In an example given by Voroney et al. (1991), to detect a difference of 20 counts/min (cpm) at a 95% confidence level, one would have to count a sample containing 1000 cpm for 10 min at an 85% counting efficiency. In many cases, it may not be possible to obtain a level of 1000 cpm in the LSC cocktail; using longer counting times and lower confidence intervals will enable the researcher to work with samples containing activity as low as 100 cpm in the LSC cocktail.

The ^{14}C-labeled organic material should be added at a rate not to exceed 2% of the soil weight (Jenkinson, 1971). Typically, a 100-g sieved soil sample should be weighed onto nonabsorbent waxed paper or aluminum foil. If the ^{14}C-labeled organic material to be added is a dry solid, it can be weighed and added directly to the soil and carefully mixed into the soil. Because of the potential for contamination, the operation should be completed in a fume hood. If the ^{14}C-labeled material is in aqueous solution, it can be added to the soil and thoroughly mixed. Once the labeled material is added, the soil should be adjusted to the required soil water potential by adding distilled water and the soil should be carefully mixed to ensure uniform water distribution. Excessive mixing should be avoided as puddling of the soil could result that would reduce oxygen diffusion into the soil.

The moist soil containing the ^{14}C-labeled material should be transferred into a 250-mL Erlenmeyer flask and attached to the CO_2 collection unit. Appropriate controls (soil without organic amendment) and blanks (no soil or organic amendment in the flask) should be prepared in the same manner and attached to the CO_2 collection unit. It may also be appropriate to include a soil amended with unlabeled organic material.

Several CO_2 collection units that have been used are shown in Fig. 39–1. The unit shown in Fig. 39–2 is similar to the unit given in chapter 38, Fig. 38–2. The details of the reactions involved are given in chapter 38, and the same samples used to determine the total amount of CO_2 evolved can and often are used to determine the amount of $^{14}CO_2$ evolved. One modification used for the $^{14}CO_2$ determination is that Tropaeolin O is added to the base to give it an orange color indicating a pH > 12.7. If the base

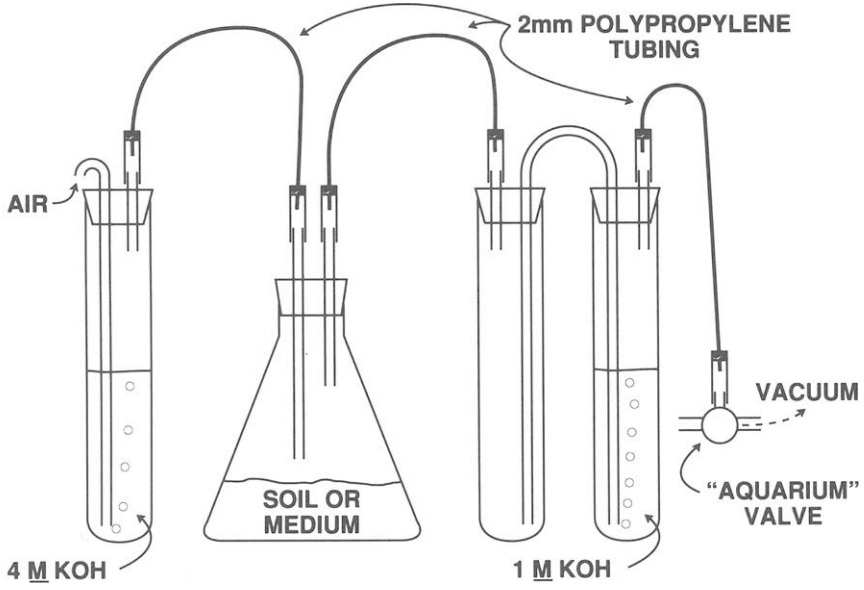

Fig. 39–2. Apparatus for studying the decomposition of ^{14}C-labeled organic materials in soil (Stevenson, 1986).

becomes partially neutralized and the pH declines to < 11, the indicator will turn yellow which indicates that the CO_2 removal efficiency has been compromised. In studies using ^{14}C-labeled materials, it is recommended that air be drawn through the system by a vacuum. Thus, if a leak develops in the system, there is little possibility of contamination of the laboratory atmosphere with $^{14}CO_2$. A leak in the system would substantially reduce the specific activity of the collected CO_2. In experiments using ^{14}C-labeled material, it is also desirable to include a secondary or backup tube to collect any $^{14}CO_2$ that might not be collected in the first tube. The air flow rate is adjusted to one bubble per second to provide adequate oxygen levels to maintain aerobic conditions. Faster rates may result in incomplete trapping of the CO_2. Gas exchange rates of 20 volumes per hour have been used (Wagner & Chahal, 1966), but a level of one volume exchange per hour is adequate in most cases. Relatively economical "aquarium" valves can be obtained at local pet supply stores and have proved durable and satisfactory.

At various intervals, the CO_2 collection tubes are replaced, and the amount of both total CO_2 evolved and $^{14}CO_2$ evolved can be determined. To determine the total CO_2 evolved, see chapter 38. To determine the $^{14}CO_2$ evolved, add an aliquot of the NaOH or KOH containing the evolved $^{14}CO_2$ to a scintillation vial containing a suitable LSC cocktail. A partial list of commercial suppliers of LSC cocktails is given in Table 39–1. New generation LSC cocktails do not contain aromatic solvents, are nonflammable, and do not result in toxic vapors. The cocktails are biodegradable and result in fewer disposal problems. The specific volumes of base

and cocktail will be determined by the load capacity of the cocktail, the activity in the sample, and the base concentration. Generally, 1 mL of 0.5 *M* NaOH or KOH can be counted using 10 mL of LSC, but the manufacturer can provide specific guidelines. For determining the radioactivity in the sample, the liquid scintillation spectrophotometer should be used according to the manufacturer's specifications. Discussions of scintillation counting procedures (L'Annunziata, 1979) and measurement of radionuclides (L'Annunziata, 1984) should be consulted.

An alternative approach is to determine the amount of activity remaining in the soil following a given incubation period. This approach generally requires combustion of the soil to convert the ^{14}C-labeled organic material to $^{14}CO_2$ and collecting the $^{14}CO_2$ from the combustion and counting it. The $^{14}CO_2$ can be collected in base and the base added to a suitable cocktail, or the $^{14}CO_2$ can be collected directly in certain cocktails and counted. Specific details are provided by L'Annunziata (1979) and Voroney et al. (1991).

39–1.3.3 Calculation of Results

Depending upon the specific scintillation spectrometer used, the results may be given as counts per unit time such as second (cps) or minute (cpm). The counts must be corrected for counting efficiency and background and historically have been given as disintegrations per unit time (dps or dpm). The SI base unit is becquerel (Bq). One becquerel is equivalent to a nuclear transformation per second, or dps. Useful conversions include 1 μCi = 37 kBq = 3.7×10^4 dps = 2.22×10^6 dpm (Corbin & Swisher, 1986). Once the appropriate dilution factors are taken into account, the percentage of the added ^{14}C evolved as $^{14}CO_2$ or remaining in the soil can be calculated (Eq. [1] and [2]). Modern-day scintillation counters allow the researcher to program the instrument to complete many of the calculations.

$$^{14}C \text{ in sample} = \frac{\text{Sample cps} - \text{Background cps}}{\text{Counting efficiency (expressed as a decimal fraction)}} \text{ (dilution factor)} \quad [1]$$

$$\%\ \text{added } ^{14}C \text{ evolved as } ^{14}CO_2 = \frac{^{14}C \text{ evolved from sample}}{^{14}C \text{ added to soil}} \text{ (100)} \quad [2]$$

39–1.3.4 Comments

It is important to report percentage of the added ^{14}C evolved as $^{14}CO_2$ or the percentage added ^{14}C remaining in the soil rather than percentage decomposition. Historically, percentage decomposition was calculated from $^{14}CO_2$ evolution data, but the amount of $^{14}CO_2$ evolved may be substantially influenced by the amount of ^{14}C incorporated into microbial

biomass or soil organic matter. The microbial products formed may be subsequently mineralized and thus influence the amount and rate of labeled substrate recovered as $^{14}CO_2$.

If, in addition to $^{14}CO_2$, volatile organics are lost from the soil and absorbed by the base used to collect $^{14}CO_2$, the amount of $^{14}CO_2$ evolved will be overestimated. Kearney and Kontson (1976) placed a polyurethane filter preceding the base trap and were able to sorb the evolved volatile products for subsequent ^{14}C determination.

Another technique for collecting $^{14}CO_2$ uses chromatographic tubes (chapter 38) that can be counted by liquid scintillation techniques. Additionally, Mayaudon (1971) presents a detailed discussion of radiorespirometry techniques in soil systems. Also, a direct soil counting technique has been used for determining ^{14}C remaining in soil treated with an herbicide (Lavy, 1975; Scott & Phillips, 1972).

39–2 ^{13}C NATURAL ABUNDANCE TECHNIQUE: BACKGROUND AND PRINCIPLES

39–2.1 Introduction

Approximately 98.89% of all C in nature is ^{12}C, and 1.11% is ^{13}C. The relative proportions of these two stable isotopes in nature vary slightly around these average values as a result of isotopic fractionation during physical, chemical, and biological processes (Boutton, 1991b). The $^{13}C/^{12}C$ ratio of organic C found in terrestrial environments is determined largely by the C isotope fractionation that occurs during photosynthesis. Plants with the C_3 photosynthetic pathway exhibit greater discrimination against ^{13}C than plants with the C_4 pathway. These natural isotopic differences between plants can be used to study the dynamics of organic matter in soil.

39–2.1.1 Stable Isotope Terminology

Because natural variation in the ratio of $^{13}C/^{12}C$ is small, stable C isotope ratios are expressed in relative terms as $\delta^{13}C_{PDB}$ values:

$$\delta^{13}C_{PDB}\ (‰) = \left[\frac{R_{sample} - R_{PDB}}{R_{PDB}} \right] \times 10^3 \qquad [3]$$

where R_{sample} is the mass 45 ($^{13}C\ ^{16}O\ ^{16}O$) to mass 44 ($^{12}C\ ^{16}O\ ^{16}O$) ratio of the sample and R_{PDB} is the $^{13}C/^{12}C$ ratio of the international PDB limestone standard, which has a value of 0.0112372 (Craig, 1957). The PDB standard was a *Belemnitella americana* limestone fossil from the Cretaceous Pee Dee formation in South Carolina. Corrections are made for the presence of ^{18}O and ^{17}O in the CO_2. Thus, $\delta^{13}C_{PDB}$ is a relative index that indicates the parts per thousand (per mil, or ‰) difference between the $^{13}C/^{12}C$ ratio of the sample and that of the PDB standard. For example,

a $\delta^{13}C_{PDB}$ value of −10 °/oo indicates a sample with a $^{13}C/^{12}C$ ratio 10 parts per thousand lower than the PDB standard; a $\delta^{13}C_{PDB}$ value of +5 °/oo indicates a sample with a $^{13}C/^{12}C$ ratio 5 parts per thousand greater than the PDB standard.

39–2.1.2 Stable Carbon Isotope Ratios of Plants and Soils

The ^{13}C natural abundance technique for studying soil organic matter dynamics uses natural differences in $\delta^{13}C_{PDB}$ values between plants with the C_3 and C_4 pathways of photosynthesis. Atmospheric CO_2 has a $\delta^{13}C_{PDB}$ value of approximately −8 °/oo (Levin et al., 1987). During photosynthesis, plants with the C_3 pathway discriminate against atmospheric $^{13}CO_2$ to a greater extent than C_4 plants (O'Leary, 1988). The C_3 plants have $\delta^{13}C_{PDB}$ values ranging from approximately −32 to −20 °/oo (mean = −27 °/oo), while C_4 plants have $\delta^{13}C_{PDB}$ values ranging from −17 to −9 °/oo (mean = −13 °/oo). Thus, C_3 and C_4 plants have distinct stable C isotope ratios and differ from each other by approximately 14 °/oo on average (Smith & Epstein, 1971). Plants with Crassulacean acid metabolism (CAM) usually have $\delta^{13}C_{PDB}$ values typical of C_4 plants; however, under certain environmental and developmental circumstances, some CAM species are able to switch to a C_3 mode of photosynthesis. These "facultative" CAM species will have $\delta^{13}C_{PDB}$ values depending upon the relative proportions of C fixed via CAM and C_3 modes. The CAM plants have $\delta^{13}C_{PDB}$ values ranging from approximately −28 to −10 °/oo, but are most commonly −20 to −10 °/oo.

Most terrestrial plant species are C_3. Most temperate zone and all forest communities are dominated by C_3 species. However, C_4 and CAM plants are significant components of many plant communities, particularly in warm, arid, or semiarid environments (Osmond et al., 1982). For example, tropical and subtropical grasslands consist almost exclusively of C_4 grasses, and CAM plants (e.g., Cactaceae, Euphorbiaceae) are important in many desert communities. In general, the proportion of C_4 species in a flora increases as latitude and altitude decrease (e.g., Teeri & Stowe, 1976; Boutton et al., 1980).

Although there are small isotopic differences between different parts of the same plant (up to 2 °/oo different from the whole plant) and between specific biochemical fractions within plants (up to 8 °/oo different from the whole plant), the C isotopic signature of the whole plant is largely preserved as dead plant tissue decomposes and enters the soil organic matter pool (Nadelhoffer & Fry, 1988; Melillo et al., 1989). Thus, soil organic matter in C_4 plant communities will have $\delta^{13}C_{PDB}$ values near −13 °/oo, while organic matter from soils in C_3 communities will be near −27 °/oo. This natural isotopic "label" in the soil organic matter enables reconstruction of the prior history of plant communities (Dzurec et al., 1985) and also permits estimation of soil organic matter dynamics in situ over relatively long periods without any type of experimental disturbance.

39–2.1.3 Natural ^{13}C and Measurement of Organic Matter Dynamics

If a community dominated by C_3 plants has been compositionally stable for a relatively long time (e.g., 500–1000 yr), then the soil organic matter in that community is in isotopic equilibrium with that C_3 vegetation; that is, they should have approximately the same $\delta^{13}C$ values. If that C_3 community (e.g., a forest) is converted to a C_4 plant community (e.g., a corn field or tropical grass pasture), then the isotopic composition of the soil organic matter will begin to shift towards that of C_4 vegetation as the C_3 component decays out of the soil and is replaced by C_4 organic matter inputs. The rate at which the original mass of C_3-derived organic matter (which is uniquely and readily identifiable by its characteristic $\delta^{13}C$ value) decays out of the system through time is a direct measure of the turnover rate of organic matter in that system. It should be noted that cultivation of the soil will accelerate organic matter turnover. Thus, changing the $\delta^{13}C$ value of the organic matter inputs (i.e., from $C_3 \rightarrow C_4$, or from $C_4 \rightarrow C_3$) is equivalent to in situ labeling of the soil organic matter (Balesdent et al., 1987). Measurements of turnover rates using the ^{13}C natural abundance technique are best suited to time periods of tens to thousands of years because: (i) the C isotopic difference between C_3 and C_4 plants is relatively small; and, (ii) the mass of existing soil organic C derived from the previous vegetation is large relative to the annual increments of organic C derived from the new vegetation (Balesdent et al., 1988). However, significant differences in $\delta^{13}C$ have been detected in upper A horizons in as little as 3 mo following a single input of C_4 litter into a C_3 plant system (Insam et al., 1991).

The use of the ^{13}C natural abundance technique obviously will be limited to situations where there has been a change from $C_3 \rightarrow C_4$ or $C_4 \rightarrow C_3$ vegetation. However, these situations are common and have been used to study organic matter dynamics where: (i) C_3 rain forest has been converted to C_4 pasture or C_4 crops; (ii) C_4 grassland has been converted to C_3 crops; (iii) C_4 savanna has been converted to C_3 woodland; and (iv) C_3 cropland has been converted to C_4 cropland (Balesdent et al., 1987, 1988, 1990; Vitorello et al., 1989; Martin et al., 1990; Skjemstad et al., 1990; Cerri et al., 1991).

39–2.2 ^{13}C Natural Abundance Technique: Methodology

39–2.2.1 Special Apparatus

1. Vacuum manifold capable of achieving 10^{-3} torr for evacuating and sealing combustion tubes.
2. Gas-oxygen torch for making quartz combustion tubes and sealing the tubes after being loaded with sample and evacuated.
3. Shade 8 or darker welding goggles to protect eyes while heating quartz with gas-oxygen torch.

4. High vacuum system capable of 10^{-3} torr for cryogenic separation and purification of CO_2 produced during combustion of organic matter in sealed quartz tubes.
5. Analytical balance readable to 0.01 mg.
6. Programmable muffle furnace.
7. Dewar flasks for holding liquid N or dry ice slush during CO_2 isolation procedure.
8. Nier-type, dual inlet, triple collector gas isotope ratio mass spectrometer for measuring ratios of isotopic species of CO_2 produced by combustion of organic matter.

39–2.2.2 Reagents

1. HCl (0.5 *M*) to volatilize carbonate C from soils containing pedogenic or lithogenic carbonates.
2. NaCl, NaI, $ZnBr_2$, or CsCl to produce high density (1.2–1.8 g cm^{-3}) liquid for isolation of undecomposed particulate organic debris in soils.
3. Quartz or vycor tubing (9 mm o.d. × 7 mm i.d.) for combustion of soil organic matter samples (Quartz Scientific, Fairport Harbor, OH).
4. Wire-form CuO with a low C background suitable for microanalysis (Fisher Scientific C474-500 or equivalent).
5. Reduced Cu granules, −10 to +40 mesh (Aldrich Chemical Co. catalog no. 31,140-5 or equivalent).
6. Quartz wool.
7. Liquid N.
8. Ethanol-dry ice slush (−78 °C).
9. Carbon isotope standards calibrated relative to the international PDB standard (available from NIST, Gaithersburg, MD, or from IAEA, Vienna, Austria).

39–2.2.3 Procedure

39–2.2.3.1 Field Sampling. The study site must consist of an area known to have been converted from a C_3-dominated to a C_4-dominated plant community (or vice versa) at a precisely known time, and there should be a remnant of the original plant community nearby to provide baseline samples. Ideally, one would like to sample sites on the same soil and in close proximity to one another that have undergone the same type of conversion at different times. Sampling such a chronosequence would provide detailed kinetics of the turnover process. To characterize the isotopic composition of the organic matter inputs, live plant tissue as well as litter should be sampled from both the baseline site with the original vegetation and the derived site with the new plant community. Plant tissue should be dried at 70 °C, ground to pass a 0.4-mm (40-mesh) screen, and set aside for isotopic analysis.

Soil samples can be obtained either by digging a pit or taking cores. Pit sampling reveals soil structure and horizonation more readily than cores, does not compress the profile, and enables more accurate sampling for bulk density. Sampling depth intervals according to horizonation enables $\delta^{13}C$ to be related to pedogenesis, and bulk density measurements at several depths in the profile will allow C dynamics to be expressed on Mg C/ha basis (1 Mg = 10^6g). Samples should be taken from several depth intervals to a depth of approximately 1 m. Samples from several pits or cores can be bulked to obtain representative $\delta^{13}C$ values of a large area or kept separate for analysis of spatial variability.

39–2.2.3.2 Preparation of Soil Samples. Soil samples should be passed through a 2-mm screen and rocks and large roots removed. Undecomposed particulate organic debris, or the "light fraction" (Stevenson & Elliott, 1989), is removed from the soil samples by flotation in high density (1.2–1.8 g cm^{-3}) inorganic solutions. Suitable inorganic chemicals for the preparation of high density liquids include NaCl, NaI, $ZnBr_2$, CsCl, and Na metatungstate. Approximately 100 g of soil is added to a 600-mL beaker, and the beaker is filled with a saturated NaCl solution (density $\cong$ 1.2 g cm^{-3}). The soil is stirred vigorously, and particulate organic debris floats to the surface. After the soil settles, the organic debris can be siphoned or strained off the surface of the liquid. By repeating this process five times, virtually all particulate organic debris is removed from the soil, and the removal can be verified by examining the soil with a dissecting microscope. The particulate organic debris, which is largely roots, can be pooled with the larger roots removed by sieving, treated with 0.5 *M* HCl to remove any adhering carbonate C, dried at 70 °C, ground to pass a 0.4-mm (40-mesh) screen, and set aside for isotopic analysis. The NaCl is then removed from the root-free soil by repeated washing in distilled water. Failure to remove residual salt may interfere with determination of organic C content later in the procedure.

If soils have pedogenic or lithogenic carbonates present, this C must be destroyed. Although the $\delta^{13}C_{PDB}$ value of pedogenic carbonate is related to the C_3 to C_4 composition of the plant community, both pedogenic and lithogenic carbonate are significantly enriched in ^{13}C and would seriously confound stable C isotope measurements of the soil organic matter. Approximately 100 g of soil is placed in a 600-mL beaker which is then filled with 0.5 *M* HCl and stirred. Soil is left in the 0.5 *M* HCl for 3 d, and the HCl solution is replaced daily. When carbonates have been removed, soils are washed repeatedly in distilled water to remove excess HCl. This acid pretreatment to eliminate carbonates has no effect on either the organic C content of the soil, or on the $\delta^{13}C$ value of the soil organic C (Boutton et al., unpublished data).

If turnover of only the bulk soil organic matter is to be measured, then the soils can be dried, ground to pass a 0.5-mm screen, and set aside for isotopic analysis. However, because different organic matter fractions exhibit different turnover rates, most investigators now choose to process soil

samples into more defined categories such as particle-size fractions, aggregate size classes, or the classical humic fractions. The most useful information is gained when biologically significant fractions are analyzed. In some studies, particle-size fractionation has been followed by extraction of specific humic substances from the particle-size fractions. Others have described in detail the methods for the isolation of particle-size fractions (Jackson, 1969; as modified by Tiessen & Stewart, 1983), aggregate size fractions (Kemper & Rosenau, 1986), and the humic fractions (Schnitzer, 1982). Following any of these more detailed procedures, the soil organic matter fractions must be dried and ground to pass a 0.5-mm screen prior to isotopic analysis.

39–2.2.3.3 Conversion of Organic Carbon to Carbon Dioxide for Mass Spectrometry. Due to instrumental requirements, C must be converted to CO_2 for stable isotope ratio measurements by mass spectrometry. The most common and simplest method to convert organic C to CO_2 is by combustion with an excess of CuO in an evacuated, sealed quartz tube at 850 °C (Boutton, 1991a). This method does not change the C isotope composition of the original sample, produces quantitative yields of C (which permit determination of percentage C in the sample), and is rapid and relatively inexpensive.

Quartz or vycor tubing (9 mm o.d. × 7 mm i.d.) is cut to 20-cm lengths, and the tubes are sealed at one end with a gas-oxygen torch. When heating quartz or vycor to the softening point, always work under a fume hood to exhaust the resulting toxic gases and wear quartz-working goggles (Wale Apparatus, Hellertown, PA) or shade 8 or darker welding goggles to protect eyes from intense glare. The prepared tubes sealed on one end only, a porcelain crucible containing the wire-form CuO catalyst (e.g., Fisher Scientific, catalog no. C474-500), and another crucible containing quartz wool are then heated in a muffle furnace at 850 °C for 1 h to remove potential organic contaminants. Upon cooling, the CuO and quartz wool can be stored separately in clean jars and the combustion tubes stored in a desiccator until ready for use.

On a piece of weighing paper, weigh out 1.0 g of CuO catalyst. Then, tare the balance and weigh out enough sample to provide approximately 2 to 3 mg of C. If this step can be carried out on an analytical balance readable to 0.01 mg or on a microbalance, the percentage C in the sample can be determined later. The amount of soil required to provide 2 to 3 mg of C will vary mostly as a function of depth in the profile. Plant tissue, roots, and litter usually contain 40 to 50% C, and a 4- to 5-mg sample of these materials is adequate to provide 2 to 3 mg of C. Too much C can result in explosion of tubes during combustion. When the appropriate amount of sample has been weighed, mix the sample and CuO thoroughly, and use a long-stem funnel to deliver the sample/CuO mixture to the bottom of a pre-combusted quartz tube. To hold the soil in place during evacuation of the tubes later, a plug of pre-combusted quartz wool can be inserted into the combustion tube and positioned above the soil/CuO mix-

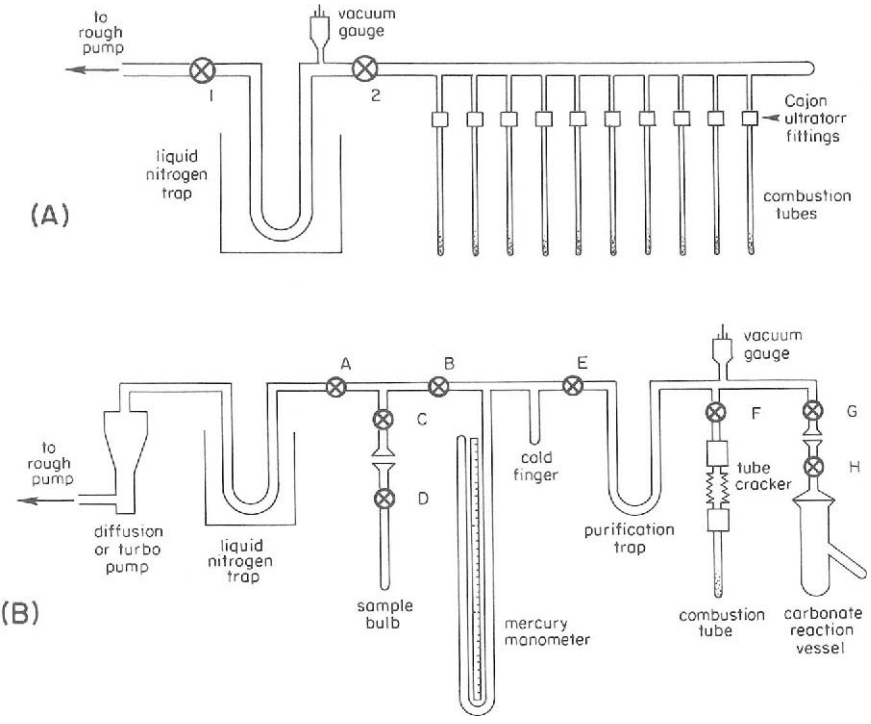

Fig. 39–3. Vacuum systems for sealing quartz combustion tubes under vacuum (A), and for isolation and volumetric measurement of CO_2 produced by combustion of organic matter (B). Circles with x's represent valves. Reproduced from Fig. 2 in Boutton (1991a), with permission from Academic Press.

ture. A quartz wool plug is not necessary for plant tissue, roots, or litter samples. Again using the long-stemmed funnel, add 0.5 g of reduced Cu granules (e.g., Aldrich Chemical Co., catalog no. 31,140-5) to the combustion tube. The long-stem funnel should be cleaned thoroughly between samples. Finally, the sample tubes must be identified clearly with an engraving tool or a high temperature marking pen capable of resisting 900 °C.

Combustion tubes loaded with samples are attached to a vacuum manifold (Fig. 39–3a) with Cajon Ultratorr O-ring fittings (Cajon Company, Macedonia, OH). Samples should be exposed to the vacuum slowly to avoid sucking the samples out of the tubes. When a vacuum of $< 10^{-2}$ torr has been achieved, the combustion tubes can be sealed with a gas-oxygen torch. Sealed tubes are then placed inside individual ceramic or inconel tubes in the muffle furnace to shield each tube from possible explosions during combustion.

The muffle furnace is heated to 900 °C and held at that temperature for 2 h. During this time, organic C is oxidized to CO_2. After 2 h, the muffle furnace is cooled to 650 °C, maintained at that temperature for two

additional hours, and then cooled to room temperature. If a programmable muffle furnace is not available, it is acceptable to simply turn off the furnace after 2 h at 900 °C, and allow it to cool slowly to room temperature. While the furnace is at 650 °C, the reduced copper granules eliminate halogens, and catalyze the conversion of any CO to CO_2, NO_x to N_2, and SO_x to $CuSO_4$ (Frazer & Crawford, 1963). Since NO_2 (mass 46) and N_2O (mass 44) have the same masses as isotopic species of CO_2, it is critical that these gases be eliminated prior to mass spectrometric analysis. High resolution mass spectrometric analysis of the gases produced by combustion of several different organic compounds by this method have revealed the presence of only CO_2, H_2O, and N_2 (Boutton et al., 1983). Sealed tubes that have been combusted should not be stored for more than 5 d prior to isolation of CO_2 because carbonate forms slowly and $\delta^{13}C_{PDB}$ of the CO_2 decreases by 1 to 3 °/oo after 2 wk (Engel & Maynard, 1989). If tubes are stored for more than 5 d, the problem can be avoided simply by recombusting the tubes prior to CO_2 isolation and purification (Engel & Maynard, 1989).

Prior to isotopic analysis, the CO_2 produced by combustion must be isolated from the other combustion products and purified by cryogenic distillation. A vacuum system for this purpose is shown in Fig. 39–3b. When operating this vacuum system or handling combusted quartz tubes, safety glasses should be worn to protect against explosions, implosions, or cryogenic liquids. The combustion tube is scored at one end and inserted into the tube cracker (Des Marais & Hayes, 1976), a sample bottle is attached to the manifold, all valves are opened, and the entire vacuum system is pumped down to $< 10^{-3}$ torr. Then, a Dewar flask containing liquid N (−196 °C) is placed around the purification trap, and valve E is closed. The top of the combustion tube is broken off by flexing the tube cracker, and the gases produced during combustion are released into the vacuum system; water and CO_2 freeze into the purification trap. After 4 min, valve F is closed, valve E is opened, and all noncondensible gases (mostly N_2) are pumped away. When the vacuum is restored to 10^{-3} torr, valve B is closed, and the liquid N Dewar flask is removed from the purification trap and replaced with a Dewar containing an ethanol-dry ice slush (−78 °C). The CO_2 sublimes but water remains frozen in the trap. To measure the volume of CO_2 produced from the organic matter, the CO_2 is transferred into the manometer cold finger by cooling it with a Dewar of liquid N; 2 min should be allowed for the CO_2 to freeze into the cold finger. When the transfer is complete, valve E is closed, the liquid N Dewar is removed from the cold finger, and the CO_2 is allowed to expand into the mercury manometer calibrated previously with known volumes of CO_2. The manometer reading is noted and, together with the weight of the sample combusted, is used to calculate the percentage C of the sample. Valve A is then closed, and valves B, C, and D are opened. The CO_2 is transferred into the sample bulb by immersing it in a liquid N Dewar for 2 min. When the transfer is complete, valves C and D are closed, and the

sample bulb is detached from the vacuum system and attached to the inlet system of the mass spectrometer.

An electronic capacitance manometer could be used in place of the mercury manometer shown in Fig. 39–3b. The capacitance manometer is more expensive but eliminates the need for mercury and is significantly more accurate for determining percentage C. Accuracy for electronic manometers ranges from 0.15 to 1% of reading, depending upon the model, while that for a mercury manometer ranges from approximately 2 to 5% of reading.

Organic matter can be combusted to CO_2 in Pyrex tubing at 550 °C (Sofer, 1980; Vitorello et al., 1989) with procedures identical to those outlined above. The primary advantage of this alternative is that Pyrex tubing is approximately 5 to 10% of the cost of quartz tubing. If Pyrex is used, great care should be taken to ensure good contact between sample and CuO, and combustion time should be increased to 12 h. Some reports indicate that accuracy and precision of $\delta^{13}C_{PDB}$ values obtained by combustion in Pyrex tubes at 550 °C are poorer than those obtained with quartz at 850 °C (Boutton et al., 1983; Le Feuvre & Jones, 1988; Swerhone et al., 1991). Furthermore, combustion at 550 °C may not give quantitative yields of CO_2, eliminating the possibility of determining percentage C during CO_2 isolation and purification (Boutton et al., 1983).

39–2.2.3.4 Mass Spectrometric Analysis and Isotopic Indices. Stable C isotope ratios are measured on the CO_2 generated by the above procedure with a dual-inlet, triple-collector gas isotope ratio mass spectrometer. The high precision of these instruments is due to simultaneous collection of the ion beams (masses) of interest and to repeated measurements of sample and standard gases by alternate switching during a single isotope ratio determination. The theory and methods of determining the isotopic composition of CO_2 by mass spectrometry have been reviewed (Craig, 1957; Deines, 1970; Mook & Grootes, 1973; Gonfiantini, 1981; Santrock et al., 1985). The $\delta^{13}C_{PDB}$ value is determined using Eq. [3].

Most of the error in isotopic measurements results from sample preparation. Mass spectrometer precision (1 SD), as determined by repeated analyses of the same gas sample, is often as low as 0.01 °/oo. By contrast, different preparations of aliquots of the same sample generally will have a precision (1 SD) of 0.1 °/oo for both plants and soils.

The PDB standard was derived from a limestone of marine origin with an absolute $^{13}C/^{12}C$ ratio of 0.0112372 (Craig, 1957). As the basis of the PDB scale, it has a $\delta^{13}C_{PDB}$ value of 0 °/oo. The PDB standard no longer exists, but several other primary standards were calibrated against it before the supply was exhausted, so it is still possible to express $\delta^{13}C$ values relative to PDB. Primary C isotope standards are available from the National Institute of Standards and Technology (NIST, formerly the National Bureau of Standards or NBS) or from the International Atomic Energy Agency (IAEA) in Vienna, Austria.

For some mass balance calculations, it is more appropriate to use the absolute ratio (R) or the fractional abundance (F) of ^{13}C in a sample. The absolute ratio is calculated by rearrangements of Eq. [3]:

$$R_{sample} = {}^{13}C/{}^{12}C = \left[\frac{\delta^{13}C_{sample}}{1000} + 1 \right] \times R_{PDB} \quad [4]$$

where $R_{PDB} = 0.0112372$. The fractional abundance is the fraction of total C in a sample that is ^{13}C:

$$F = \frac{{}^{13}C}{{}^{13}C + {}^{12}C} = \frac{R_{sample}}{R_{sample} + 1} \quad [5]$$

Additional details on these indices and their relationship with $\delta^{13}C_{PDB}$ are provided by Hayes (1983).

39–2.2.3.5 Calculating Sources of Soil Organic Matter. The relative proportions of soil C derived from C_3 and C_4 sources can be determined by simple mass balance calculations. Assuming that we have a situation where a C_4 plant community has replaced a C_3 community, then the proportion of C (p) derived from the C_4 community at some later point in time (t) can be calculated as:

$$F = (p)F_{C4} + (1\text{-}p)\ F_{C3} \quad [6]$$

where F is the fractional abundance (see Eq. [5]) of the soil organic matter fraction of interest at time t after the transition from $C_3 \rightarrow C_4$, F_{C4} is the fractional abundance of the C_4 organic matter inputs (often an average of shoot, roots, and litter), F_{C3} is the fractional abundance of the soil organic matter fraction of interest prior to the change in vegetation, and 1-p is the proportion of C_3 plant-derived C still present in the soil at time t. Since the $\delta^{13}C_{PDB}$ scale is not linear, it is technically more correct to use fractional abundance (F) as an isotopic index in mass balance calculations such as Eq. [6]; however, over the range of $\delta^{13}C_{PDB}$ values encountered in plant-soil systems at natural abundance, $\delta^{13}C_{PDB}$ is sufficiently linear that Eq. [6] can be rewritten as:

$$\delta = (p)\ \delta_{C4} + (1\text{-}p)\ \delta_{C3} \quad [7]$$

where $\delta^{13}C_{PDB}$ values have been substituted for the fractional abundances used in Eq. [6]. Equation [7] can be rearranged and simplified to:

$$p = \frac{\delta - \delta_{C3}}{\delta_{C4} - \delta_{C3}} \quad [8]$$

Since the percentage C of each of the organic matter fractions was measured above as part of the sample combustion procedure outlined earlier, the relative proportions of C from C_4 (p) and C_3 (1-p) sources can be used to compute the actual masses of C from each organic matter source. If the total mass of C M (with units of mg C/g soil) is known, then the mass of C from C_4 vegetation (M_{C4}) can be calculated as:

$$M_{C4} = M\ (p) \qquad [9]$$

Similarly, the mass of C from C_3 vegetation (M_{C3}) can be calculated as:

$$M_{C3} = M\ (1\text{-}p) \qquad [10]$$

Thus, if the study site consists of plots that have been switched from C_3 to C_4 at different times or if multiple times are available from the same site, the decay of the C_3 C out of the soil system and the rate of entry of C_4 C into the soil system can be described with respect to time (Balesdent et al., 1987; Skjemstad et al., 1990; Andreux et al., 1990; Cerri et al., 1991).

If soil bulk density measurements are taken, it is possible to convert the data acquired above into C content per unit area:

$$\text{Mg C/ha} = (\text{g C/g soil}) \times \varrho_b \times l \times 10^4 \qquad [11]$$

where ϱ_b is the bulk density of the soil layer under consideration (Mg soil/m^3), and l is the thickness (meters) of the soil layer under consideration. Carbon dynamics for the entire soil can be determined by solving Eq. [11] for each depth interval (e.g., 0–0.1 m, 0.1–0.2 m, etc.) and then summing the results.

Although we have assumed a situation where a C_4 plant community has replaced a C_3 community, the calculations for the reverse situation ($C_4 \rightarrow C_3$) are directly analogous to those described above.

39–2.2.3.6 Kinetics of Soil Organic Matter Turnover. Although many complex processes are responsible for soil organic matter turnover, it is generally accepted that the overall process can be described reasonably well according to first order rate kinetics which assume a single homogeneous pool of soil organic C (Jenkinson & Rayner, 1977; Paul & van Veen, 1978; Paul & Clark, 1989). When studying organic matter dynamics where a C_4 community has replaced a C_3 community, the decay of C_3 C out of the system can be approximated by the negative exponential or first order decay model:

$$A_t = A_o\, e^{-kt} \textit{ or } \ln (A_t/A_o) = -kt \qquad [12]$$

where A_t is the mass of C_3-derived C at some time t after the $C_3 \rightarrow C_4$ switch, A_o is the mass of C_3-derived C at time 0, k is the fractional rate constant with units of time^{-1}, and t is the length of time elapsed since the

$C_3 \rightarrow C_4$ switch. The value of the fractional rate constant k is equal to the slope of the line obtained by plotting ln (A_t/A_o) against time. The half-life $(t_{1/2})$ of C_3-derived C in the system is then equal to 0.693/k, and the mean residence time or turnover time is equal to $-1/k$ (Paul & Clark, 1989). Because of the poor fit of a single compartment model to most experimental results, more complex multiexponential (multicompartmental) models would permit representation of the kinetics of labile and recalcitrant pools of soil C (e.g., Andreux et al., 1990).

39–2.2.4 Comments

The ^{13}C natural abundance technique for measuring soil organic matter dynamics is complementary to the tracer approaches using ^{14}C- or ^{15}N-enriched organic matter. Natural ^{13}C allows work to be done on large spatial scales as opposed to small plots or pots, it involves minimal perturbation to the system, and it can elucidate kinetics over relatively long periods of tens to hundreds of years. Furthermore, this technique usually operates on time scales that allow all soil organic matter pools, even those that are recalcitrant, to become "labeled." In tracer experiments with ^{14}C or ^{15}N, the duration of the experiments (usually < 5 yr) is such that the more recalcitrant fractions may not become labeled. Because this technique involves only naturally occurring stable isotopes of C, there are no hazards, regulations, or disposal problems associated with its use.

The technique also has some weaknesses that should be addressed. One of the major problems is that it is difficult to measure the exact $\delta^{13}C_{PDB}$ value of the organic matter inputs following the vegetation change (e.g., δ_{C4} in Eq. [8]). The $\delta^{13}C_{PDB}$ values of plant tissue vary slightly in response to environmental conditions and can show small differences between plant parts and between biochemical fractions. However, $\delta^{13}C_{PDB}$ values of litter samples integrated through time should serve as a reasonable estimate of the isotopic composition of the organic matter input.

Another potential problem is that, in well-drained mineral soils that have supported a stable plant community for a long time, $\delta^{13}C_{PDB}$ of the soil organic matter increases by 1 to 2 °/oo from the surface to approximately 1 m in depth (Stout et al., 1981). It has been suggested that this ^{13}C-enrichment is a consequence of microbial metabolism, with microbes typically being slightly more enriched in ^{13}C than the substrate they grow on. Since organic matter increases in age with depth in the profile (Scharpenseel & Neue, 1984), the enrichment in the deeper layers may simply reflect the consequences of a longer history of microbial metabolism. While the natural abundance method assumes that the isotopic composition of the soil organic matter will equilibrate to the $\delta^{13}C_{PDB}$ value of the organic matter inputs of the new plant community, it is clear that the organic matter input alone does not determine the equilibrium $\delta^{13}C_{PDB}$ values of the soil. Further work is needed to elucidate the consequences of ^{13}C enrichment with soil depth on this technique.

39–3 DECOMPOSITION OF ^{15}N-LABELED ORGANIC MATTER IN SOILS

39–3.1 Introduction

A study of the decomposition of ^{15}N-labeled organic matter primarily involves the processes of immobilization and mineralization. Major aspects of these processes are covered in chapter 42. Indigenous soil organic N occurs in many complex forms that have varying mineralization rates. Much of the organic N is in a "passive" phase (Jansson & Persson, 1982) which is essentially inert over short periods. Incorporation of ^{15}N-labeled organic matter into soils permits an evaluation of the mineralization potential of newly incorporated organic N relative to that of the indigenous soil N, as well as determination of the possible size of the inert pool of soil N. Many studies of the nature and composition of soil organic matter have been carried out (Allison, 1973), but factors influencing the stability of the organic N constituents are still not clearly understood. The use of isotopic methods thus far has not enhanced our current knowledge of the stabilizing factors, but information has been obtained concerning the rate at which newly incorporated N becomes stabilized in forms similar to that of the indigenous soil organic N (Legg et al., 1971). Studies of the transformations of labeled organic N incorporated into soils have been carried out in field, greenhouse, and laboratory experiments. Although field experiments provide useful information from soils under natural conditions (e.g., Ladd & Amato, 1986), time requirements are large compared to greenhouse and laboratory studies. For that reason, emphasis will be placed on the latter two modes of study. Many variations of the described methods exist, and reference should be made to the literature cited for additional material.

Methods for the determination of mineral N are covered in chapters 41 and 42, organic N in chapter 40 and in section 39–4.3, and ^{15}N measurements are given in chapter 40. Additional discussions of methods are presented by Bremner and Mulvaney (1982), Keeney and Nelson (1982), Hauck (1982), and Mulvaney (1993).

39–3.2 Labeling Organic Matter with ^{15}N

Generally, the method selected for labeling organic matter will depend upon the type and purpose of the experiment. The methods may be categorized as (i) labeling plants by supplying growing plants with ^{15}N-labeled fertilizer or (ii) labeling soil biomass by supplying soil microorganisms with ^{15}N plus an energy source (see also chapter 40). In any case, the enrichment with ^{15}N should be sufficient to be easily detectable in the total soil N. This depends upon the amount of ^{15}N added, its enrichment, and the total N content of the soil. The minimum amount of N per sample for many mass spectrometers is about 0.5 to 1 mg. It is useful to have an excess ^{15}N percentage of 0.500 or more if the total N of the sample is so small that

an addition of a measured amount of unlabeled $(NH_4)_2SO_4$ is needed to meet the minimum N requirement. Calculation of results from this dilution technique has been covered by Hauck (1982).

39–3.2.1 Growing Plants with ^{15}N-Labeled Fertilizer

39–3.2.1.1 Growing Plants in Soil to be Labeled. A greenhouse pot experiment may be set up in which ^{15}N-labeled fertilizer is added to the soil, plants are grown, and harvested plant material is chopped or ground and returned to the soil. This process can be repeated as many times as desired, provided a short incubation period is allowed between crops so that some N is mineralized to reduce N deficiency in the succeeding crop. No further additions of N need be made, and additions from seed in succeeding crops can be eliminated by growing a crop, such as oat (*Avena sativa* L.), to sufficient maturity for the seed to be harvested and used for the next crop. A typical example will be given that can be modified as needed.

39–3.2.1.2 Procedure. A bulk sample of field soil (top 15–20 cm) is obtained, sieved through a 2-mm sieve to remove extraneous plant material, and mixed well. To 2-kg soil (oven-dry basis) contained in plastic bags within the pots, add 200 mg of enriched N in solution (5–10% excess ^{15}N) and other nutrient elements as needed. These materials may be easily mixed throughout the soil in the plastic bags. The form of labeled N (nitrate, ammonium, or urea) is optional. Plant 15 oat seeds in each pot of soil, cover, and water to near field capacity. Other plants can be used, but the plant must be grown to maturity so the seeds produced can be used for subsequent planting. After germination, plants may be thinned to an equal number for each pot. Return the plant material to the soil surface. During plant growth, water should be added daily by weighing the pots and adjusting the soil to field capacity. When the seed heads are sufficiently mature for germination, plants are cut at the soil surface, seeds are collected, and the stems and leaves are cut into about 2-cm pieces on a sheet of paper or plastic. The soil is removed from the pot, broken up on a plastic sheet, and roots are cut into small pieces. Soil and plant material are replaced in the plastic bag and mixed. Water is added as required for field capacity, the tops of the bags are loosely closed to reduce evaporation and allow aeration, and incubation can proceed on the greenhouse bench for 2 to 3 wk. Seeds from the pots are kept separate, allowed to air dry during the incubation period, and then used to replant the pot from whence they came.

This procedure can be repeated as many times as desired. At definite cropping intervals, triplicate pots may be removed from the experiment and analyzed. In this case, the plant material is oven dried (60 °C), weighed, ground in a Wiley mill, and analyzed for total N and atom % ^{15}N. Plant roots may be harvested, washed, and handled in the same manner. The ^{15}N-labeled soil may be used immediately or air dried and reserved for further study.

39–3.2.1.3 Growing Plants without Soil. An initial advantage of labeling plant material with ^{15}N in the absence of soil is that the ^{15}N is not diluted by the uptake of soil N. The labeled plant material can then be added to any number of soils for decomposition studies. One disadvantage is that the ^{15}N has not had the opportunity to recycle into less readily mineralized forms as in the preceding method. Sand-culture systems generally have been used to grow the plants, although solution-culture with proper aeration should also be suitable. The procedure described is similar to that used by Ladd et al. (1981).

39–3.2.1.4 Procedure. Weigh 3-kg quantities of clean, washed sand (air-dry basis) in plastic pots without drainage holes and place in the greenhouse or growth chamber. Plant seed of the desired crop and moisten the sand. After germination and thinning of plants to a suitable number, apply a nutrient solution to maintain the sand at 10% gravimetric water content. According to Gauch (1972), the nutrient solution proven satisfactory for many types of plants contains (meq/L) Ca, 10; Mg, 4; K, 4; NO_3, 10; HPO_4, 4; and SO_4, 4, with trace elements (mg/L) Cu, 0.02; Zn, 0.05; Mn, 0.5; B, 0.5; and Fe, 3 (supplied as NaFe-EDTA). The labeled nitrate (5–10 atom % ^{15}N) may be contained in the nutrient solution or added separately at intervals during the growth period. Harvest the plants at the desired stage of maturity and wash roots to remove sand. Dry the plant material and grind to the fineness needed for the soil to be labeled. For laboratory incubation studies, the plant material should be ground to pass a 1-mm sieve. For greenhouse and field studies, where large volumes of soil are involved, coarser material may be used.

39–3.2.2 Labeling Soil Biomass

Microbial biomass forms a highly important constituent of soils in that it provides for the transformation of all organic materials entering the soil, as well as being a small but labile pool of plant nutrients. Jenkinson and Ladd (1981) estimated that about 2 to 3% of the organic C in soils they examined was present as microbial biomass.

Labeling soil biomass directly, rather than through the decomposition of ^{15}N-labeled plant materials, averts any complications that might arise from the presence of labeled plant compounds resistant to decomposition. The time required for adequate labeling is also quite short (Chichester et al., 1975; Kelley & Stevenson, 1985). A simple incubation procedure with the soil to be labeled, a readily available C source, and a ^{15}N-labeled inorganic compound results in a labeled soil within a week. The procedure described is similar to that of Kelley and Stevenson (1985) but with a higher C/N ratio.

39–3.2.2.1 Procedure. Weigh 100 g of soil (oven-dry basis) that has been air dried and passed through a 2-mm sieve, into a 250-mL Erlenmeyer flask. Wet the soil with a solution containing 1.0 g of glucose C and 10 mg

of N as $(^{15}NH_4)_2SO_4$ (5 atom % ^{15}N). Incubate the soil for 1 wk at 30 °C. Remove the soil from the incubator, air dry, and store for future use. Larger soil samples may be used, or several flasks may be included in the incubation to provide enough labeled soil for the experiment.

39–3.3 Determination of Mineralization Rates

The decomposition of soil organic N is measured in terms of net mineralization rates (see chapter 42). Since the labeled organic N is not uniformly distributed throughout the organic matter of the soil, estimates of mineralized N derived from both the indigenous and labeled organic N are made. Both plant uptake studies and laboratory incubation tests have been used to determine mineralization rates. Similar results have been obtained by these two methods (e.g., Broadbent & Nakashima, 1967; Legg et al., 1971). Since plant uptake studies are more laborious and time consuming, only laboratory procedures will be outlined. It is possible to use either aerobic or anaerobic incubation to determine N mineralization (see chapter 41). In most cases, aerobic incubation has been the method of choice for the long-term, consecutive incubations and extractions that are required for organic matter decomposition studies. The aerobic system is especially useful with soil organic matter labeled with both C and N (e.g., Broadbent & Nakashima, 1974). For these reasons the aerobic system is the preferred method.

The time requirements for carrying out long-term incubations or plant uptake experiments have varied from several months to several years. Generally, it is necessary to continue tests until the relative amounts of mineralized N derived from indigenous and labeled organic sources become stabilized. The calculated "availability ratios" (Broadbent & Nakashima, 1967) vary among soils, but the reasons for such variations have not been elucidated. The described procedure essentially follows that of Broadbent and Nakashima (1967) and allows determinations of mineralization rates at regular intervals for extended periods. Size of the soil sample to be incubated may vary, depending upon the expected mineralization rate.

39–3.3.1 Procedure

Weigh out triplicate 50-g samples of labeled soil (oven-dry basis) on paper sheets, moisten, mix, and transfer to leaching tubes with fritted glass bottoms. By moistening and mixing the soil, the finer particles will not segregate out and form layers in the tubes that may impede leaching. For clayey soils that are not easily leached, it may be necessary to mix the soil with sand or expanded vermiculite to facilitate leaching. A thin layer of glass wool at the top of the soil column will reduce the dispersive action of the leaching solution on the soil particles. The leaching tubes are fitted with rubber stoppers suitable for use on small suction flasks.

Before incubation, leach the soil tubes with 80 mL of saturated $CaSO_4$ solution, applied in four equal aliquots, to remove initial mineral N. Remove excess solution with suction, and incubate the samples at 35 °C in a humid atmosphere for 2 wk. Transfer the leachate to semi-micro Kjeldahl flasks and determine the inorganic N by distillation with MgO and Devarda's alloy (see chapter 40). Alternatively, the leachate may be made up to 100-mL volume and aliquots taken for analysis. The ammonia from the distillation, after titration to determine the N content, is prepared for ^{15}N analysis in the mass spectrometer (chapter 40).

After the 2-wk incubation, leach the soil samples again in the prescribed manner and determine the inorganic N and ^{15}N content of the leachate. Any number of incubations may be carried out to obtain a pattern of the organic N decomposition that is occurring. If the amount of N mineralized in 2 wk becomes insufficient for ^{15}N analysis, the incubation period may be extended, with precautions taken for maintaining the water content of the soil.

At the conclusion of the incubation and leaching part of the experiment, remove soil from the leaching tubes and dry and prepare it for total N analysis (see chapters 40 and 41) as well as ^{15}N content. A comparison of the data for the original soil and that for the residual soil plus mineralized inorganic N will indicate whether any appreciable undetermined loss of labeled N has occurred during the prolonged incubation period.

The above procedure can be modified in many ways, such as size of soil sample and different leaching solutions. Quite often, adaptations are easily made to accommodate the laboratory equipment available without any sacrifice in precision of results.

39–3.4 Preparation of Samples for ^{15}N Analysis

The general procedures involved in ^{15}N analysis will not be given in this chapter. Refer to chapter 40, Hauck (1982), and Mulvaney (1993) for details. Each mass spectrometry laboratory has developed its own special apparatus for preparing and analyzing ^{15}N samples; therefore, it is necessary for anyone unfamiliar with a particular mass spectrometry laboratory to determine the most appropriate form of the research samples.

39–3.5 Calculations

The "availability ratio" concept developed by Broadbent and Nakashima (1967) has been found useful in both plant uptake and mineralization studies to determine the relative mineralization rates of indigenous and ^{15}N-labeled organic N (e.g., Chichester et al., 1975; Legg et al., 1971). For extracts of mineral N after incubation, the equation is as follows:

$$\text{Availability ratio} = \frac{\text{Labeled N (extract)} / \text{Total N (extract)}}{\text{Labeled N (soil)} / \text{Total N (soil)}} \quad [13]$$

If the ^{15}N-labeled soil organic N has the same availability to microorganisms as the indigenous organic N, the availability ratio will be one. On the other hand, if the labeled N is more susceptible to mineralization than the indigenous N, the availability ratio will be greater than one. A ratio of less than one is conceivable, but not likely.

The component values in the equation are those at the beginning of each incubation; therefore, in a succession of incubations, the values for labeled and total N in the soil must be corrected to account for the N mineralized and extracted in the previous incubation. This correction assumes that no other losses of any consequence have occurred. An analysis of soils after the experimental period will indicate whether such losses actually occurred.

When the organic N of soils is first labeled, the initial availability ratios obtained either by cropping or mineralization are generally high but quickly decrease to a level much closer to unity. This decrease indicates that the more labile fraction of the organic N is soon mineralized, leaving a more resistant fraction that appears to become more stable with time. The relationship between availability ratios and the degree of stability of labeled organic N incorporated into the soil has not been completely elucidated.

Other useful calculations can be made by employing the general equation for estimating quantities involved in isotopic equilibria:

$$A = B(1-y)/y \qquad [14]$$

Fried and Dean (1952) used this equation to obtain a measure of the availability of a soil nutrient, A, in terms of a given rate of a labeled fertilizer, B, where the proportion of the nutrient derived from the fertilizer, y, could be determined in the plant. The A value for N can be determined when a soil is being labeled with ^{15}N, as in section 39–3.2.1.1, by determining the fraction of total N in the plants that was derived from the fertilizer.

Jansson (1958) used the same equation in mineralization studies of recently incorporated labeled organic N. In this case, A is the amount of soil organic N in the active phase, B is the amount of newly immobilized labeled N, and y is the proportion of mineralized N after incubation that is derived from the labeled organic N. In long-term incubation or plant uptake experiments, increasing A values are generally observed. This increase reflects a stabilization of the labeled N and an equilibration with increasing amounts of soil N in a passive form.

Understanding the dynamics of residue decomposition and soil organic N turnover can be enhanced by using reaction kinetics to describe the microbial transformations involved. Paul and Clark (1989) provide a clear explanation of the mathematical equations and the utilization of specific types of data for determining zero-order, first-order, and hyperbolic reactions. Such calculations for degradation rates require relatively short-time

intervals between measurements; otherwise, microbial synthesis and soil organic matter formation may become complicating factors.

39–3.6 Comments

Studies of the decomposition of organic N in soils require several different approaches owing to the complexity of the problem, and it is not possible to cover all of them in a single chapter. Notable among these are the procedures that have been devised to extract and separate organic compounds from the soil (Schnitzer, 1982).

Some of the early work with ^{15}N described the changes taking place in the quantities of labeled N found in different extracts with time (e.g., Stewart et al., 1963). Another means of separating recently incorporated ^{15}N in soils is organomineral sedimentation fractionations (e.g., Chichester, 1970). Modeling also has become important to increased understanding of organic matter turnover and N cycle rates (e.g., Jenkinson & Rayner, 1977; Paul & van Veen, 1978; Myrold & Tiedje, 1986). Such procedures, along with the basic methods described, provide ample opportunities for even greater advances in studies of organic N decomposition.

39–4 EXTRACTION OF LABELED ORGANIC FRACTIONS IN STUDIES OF SOIL ORGANIC MATTER DYNAMICS

39–4.1 Introduction

In the preceding sections, it has been shown how the soil organic matter is labeled and how subsequent biological transformations are followed by various procedures. Much of the labeled organic matter becomes difficultly mineralizable and apparently enters stable forms similar to indigenous organic matter. Chemical extractions of soil organic matter have been used to determine the movement of labeled compounds into the various extracted fractions, as well as unknown nonhydrolyzable forms. Currently, there are no standardized procedures for separating the organic from the inorganic soil phase, and many variations in extractants, treatments, and conditions have evolved over the years. In general, the extractions involve hot mineral acids or bases, followed by separation into various fractions for analysis. Several excellent reviews cover the details of several methods for extraction and fractionation of soil organic matter (Bremner, 1965; Schnitzer, 1982; Stevenson, 1965; 1982b). As Stevenson (1965) stated:

> The great difficulty in all fractionation procedures is that the methods employed either separate out products which are not definite chemical entities, or they form artifacts which do not have the properties of the original material. Nevertheless, the various fractionation procedures have proved useful for

studying soil organic matter, and they will probably continue to be used in the future.

Quite often, different extraction procedures are used for C and N fractionations owing to differences in determinations of isotopic composition and interferences that may occur. For that reason, separate extraction procedures will be described for C and N in the following sections.

39–4.2 Extraction of Organic Matter Containing Labeled Carbon

39–4.2.1 Introduction

Numerous methods have been used to extract C-containing components found in soil organic matter. Many of the procedures have been summarized by Stevenson (1982a). The classical procedure involves alkaline extraction of the soil and precipitation of the humic acid fraction by acidification. The fulvic acid fraction is soluble in both the base and acid. Recently, substantial research has focused on determination of the fraction of organic C found in the microbial biomass that is the more biologically active and dynamic fraction of organic C found in soil. Procedures for estimating C levels present in microbial biomass are given in chapter 36.

The procedure given in the following section is a generalized scheme for the classical method of separation of humic and fulvic acid fractions found in soil organic matter. The more detailed discussions of the procedure given by Schnitzer (1982) and Stevenson (1982a) should be consulted. The procedure is easily adapted to determine the amount of ^{14}C in humic and fulvic acids as long as appropriate safety precautions are followed when working with radioactive material.

39–4.2.2 Materials

1. Soil containing ^{14}C.
2. Hydrochloric acid (HCl), 0.05 *M*, 2 *M*.
3. Sodium hydroxide (NaOH), 0.5 *M*.
4. N_2 source.
5. Horizontal or wrist-action shaker.
6. Centrifuge.
7. Polypropylene or polyethylene centrifuge tubes.
8. pH meter.
9. Dry combustion or wet oxidation unit.
10. Liquid scintillation spectrometer.
11. Liquid scintillation counting cocktail.
12. Scintillation vials.

39–4.2.3 Procedures

Weigh 40 g (dry weight equivalent) of labeled soil into a 250-mL centrifuge tube and add 200 mL of 0.05 *M* HCl. Stir the mixture with a

stirring rod. The dilute acid removes any free carbonates and polyvalent cations and increases organic matter extraction efficiency. Centrifuge and discard the supernatant. This and all subsequent centrifugations are carried out at 1500 × *g*. Add 200 mL of distilled water, stir, centrifuge, and discard the supernatant. Add 200 mL of 0.5 *M* NaOH and displace the air in the centrifuge tube with N_2. Shake the centrifuge tube for 12 to 24 h at room temperature. Separate the dark-colored supernatant that contains the humic and fulvic acids from the soil by centrifugation and decant the supernatant and filter it through glass wool to remove suspended organic solids. Collect the supernatant in a 1-L beaker. For maximum organic matter removal, repeated extraction with 0.5 *M* NaOH is required. Generally, two to three extractions are adequate for most soils. Following the alkaline extractions, add 200 mL of distilled water to the residual soil, shake for 10 min, centrifuge, and add the rinse water to the supernatant in the beaker. Add 2 *M* HCl to the alkaline extract and adjust to pH 1 using a pH meter to monitor the change. The dark-colored precipitate is humic acid and the straw-colored solution is the fulvic acid. Allow the mixture to set overnight at room temperature or refrigerate. Centrifuge the pH 1 suspension to separate the fractions. Once the humic acid has been isolated, add 200 mL of distilled water, mix, centrifuge, and add the supernatant to the fulvic acid fraction. Both fractions should be freeze-dried, weighed to determine yield, and stored in a desiccator. The residual soil contains the humin fraction and it can also be freeze-dried for subsequent analysis.

The amount of total C and ^{14}C in the humic and fulvic acids can be determined by dry combustion or wet oxidation of the organic materials. To determine the amount of total C and ^{14}C in the humin fraction, the residual soil can be analyzed. The specific details for the oxidation procedures are given by Nelson and Sommers (1982). The amount of CO_2 produced from the oxidation can be determined by methods also given in chapter 38 and the details for determining $^{14}CO_2$ levels are given in 39–1.3.2.

39–4.2.4 Comments

The humic and fulvic acid fractions will contain inorganic components or ash. Typical ash values are $\leq$10% for humic acid and $\geq$40% for fulvic acid. If the investigator is only interested in the amount of ^{14}C from the original substrate incorporated into the humic or fulvic acid fraction, it is not necessary to determine the percentage ash. However, because of the high ash contents in the extracted materials, the percentage C values will generally be much lower than values reported on a dry, ash-free basis. Procedures for purification of humic and fulvic acids have been detailed by Schnitzer (1982) and Stevenson (1982a).

Air or oven drying is not recommended for humic or fulvic acids. The best method for drying humic acid is lyophilization. Because of the large volume of liquid containing the fulvic acid, a flash evaporator is often used to concentrate the fulvic acid prior to freeze drying.

The excessive Cl^- levels in the organic matter fractions can cause problems during combustion or wet oxidation of the organic materials. It is necessary to frequently change the halide trapping units in the combustion or oxidation trains.

39–4.3 Extraction of Organic Matter Containing Labeled Nitrogen

39–4.3.1 Introduction

Classical methods for the extraction and fractionation of organic N were based upon the assumption that much of the N is proteinaceous in nature, and procedures developed for characterizing various chemical groups in proteins were used. With ^{15}N-labeled soils, such methods required certain modifications to accommodate isotopic analyses (Bremner, 1965; Cheng & Kurtz, 1963). In the modified procedure, the soil hydrolysate is neutralized without prior removal of excess acid, and the different forms of N in the neutralized hydrolysate are measured as ammonium that is readily converted to N_2 for isotope-ratio analysis. The methods described in the following sections are basically a condensed version of the ones presented by Bremner (1965) and Stevenson (1982b), and these publications should be consulted for further information. The procedure is relatively simple and permits rapid estimation of total N, ammonium N, hexosamine N, amino acid N, and (serine + threonine) N in soil hydrolysates.

39–4.3.2 Acid Hydrolysis of Soils

39–4.3.2.1 Special Apparatus

1. Micro-Kjeldahl digestion unit.
2. Steam distillation apparatus.
3. Distillation flasks: 50- and 100-mL Pyrex Kjeldahl flasks with 19/38 ground glass joints and glass hooks.
4. Microburette, 5 mL, graduated at 0.01-mL intervals.

39–4.3.2.2 Reagents

1. Sulfuric acid (H_2SO_4), concentrated.
2. Hydrochloric acid (HCl), approximately 6 *M:* Add 513 mL of concentrated HCl (specific gravity 1.19 g/cm^3) to about 400 mL of water, cool, and dilute to volume in a 1-L volumetric flask.
3. *n*-Octyl alcohol.
4. Potassium sulfate-catalyst mixture: Prepare an intimate mixture of 200 g of K_2SO_4, 20 g of $CuSO_4 \cdot 5H_2O$, and 2 g of Se. Powder the reagents separately before mixing, and grind the mixture in a mortar to powder the cake that forms.
5. Sodium hydroxide (NaOH), approximately 10 *M*.
6. Sodium hydroxide, approximately 5 *M*.

7. Sodium hydroxide, approximately 0.5 *M*.
8. Boric acid indicator solution: Place 80 g of H_3BO_3 in a 5-L flask marked at a 4-L volume, add about 3800 mL of water, and heat and swirl the flask until the H_3BO_3 is dissolved. Cool the solution and add 80 mL of mixed indicator solution. The indicator is prepared by dissolving 0.099 g of bromocresol green and 0.066 g of methyl red in 100 mL of ethanol. To the boric acid + indicator solution, add 0.1 *M* NaOH cautiously until the solution assumes a reddish purple tint (pH about 5.0), and make the solution to 4 L with water. Mix thoroughly before use.
9. Sulfuric acid, 0.0025 *M* standard.
10. Magnesium oxide (MgO): Heat heavy MgO in a muffle furnace at 600 to 700 °C for 2 h. Cool in a desiccator containing KOH pellets and store in a tightly stoppered bottle.
11. Ninhydrin: Grind 10 g of reagent grade ninhydrin in a mortar and store in a small widemouth bottle.
12. Phosphate-borate buffer, pH 11.2: Place 100 g of sodium phosphate ($Na_3PO_4 \cdot 12H_2O$), 25 g of borax ($Na_2B_4O_7 \cdot 10H_2O$), and about 900 mL of water in a 1-L volumetric flask, and shake the flask until the phosphate and borate are dissolved. Dilute the solution to 1 L and store in a tightly stoppered bottle.
13. Citric acid: Grind 100 g of reagent grade citric acid ($C_6H_8O_7 \cdot H_2O$) in a mortar, and store in a small widemouth bottle.
14. Citrate buffer, pH 2.6: Mix 2.06 g of powdered sodium citrate dihydrate ($Na_3C_6H_5O_7 \cdot 2H_2O$) and 19.15 g of powdered citric acid in a mortar, and grind to a fine powder with a pestle. Store in a small widemouth bottle.
15. Periodic acid ($HIO_4 \cdot 2H_2O$) solution, approximately 0.2 *M:* Dissolve 4.6 g of $HIO_4 \cdot 2H_2O$ in 100 mL of water and store in a glass stoppered bottle.
16. Sodium metaarsenite ($NaAsO_2$) solution, approximately 1.0 *M:* Dissolve 13 g of powdered, reagent grade $NaAsO_2$ in 100 mL of water and store in a tightly stoppered bottle.
17. Standard (NH_4^+ + amino sugar + amino acid)-N solution: Dissolve 0.189 g of $(NH_4)_2SO_4$, 0.308 g of glucosamine·HCl, and 0.254 g of alanine in water. Dilute the solution to 2 L in a volumetric flask and mix thoroughly. If prepared from pure, dry reagents, this solution contains 20 μg of NH_4^+-N, 10 μg of amino sugar-N, and 20 μg of α-amino acid-N/mL. Store the solution for no more than 7 d in a refrigerator at 4 °C.
18. Standard (serine + threonine)-N solution: Dissolve 0.150 g of serine and 0.170 g of threonine in water. Dilute the solution to 2 L in a volumetric flask and mix. If prepared from pure, dry reagents, this solution contains 10 μg of serine-N and 10 μg of threonine-N/mL. Store the solution for no more than 7 d in a refrigerator at 4 °C.

39–4.3.3 Preparation and Sampling of Soil Hydrolysate

Place a sample of finely ground (≤100 mesh) soil containing about 10 mg of N in a round-bottom flask fitted with a standard taper (24/40) ground-glass joint. Add two drops of octyl alcohol and 20 mL of 6 *M* HCl, and swirl the flask until the acid is thoroughly mixed with the soil. Place the flask in an electric heating mantle, connect the flask to a Liebig condenser fitted with a 24/40 ground-glass joint, and heat the soil-acid mixture so that it boils gently under reflux for 12 h.

After completion of hydrolysis, wash the reflux condenser with a small quantity of distilled water, allow the flask to cool, and remove the flask from the condenser. Filter the mixture through a Buchner funnel fitted with Whatman no. 50 filter paper, using a suction filtration apparatus that permits collection of the filtrate in a 200 mL tall-form beaker marked to indicate a volume of 60 mL. Wash the residue with 5- to 10-mL portions of distilled water until the filtrate reaches the 50-mL mark on the beaker. Immerse the lower half of the beaker in crushed ice, and neutralize to pH 6.5 ± 0.1 by cautious addition of NaOH, using a pH meter to follow the course of neutralization. Add the alkali slowly with constant stirring to ensure that the hydrolysate does not become alkaline at any stage of the neutralization process. Use 5 *M* NaOH to bring the pH to about 5 and complete the neutralization using 0.5 *M* NaOH. Transfer the neutralized hydrolysate by means of a small funnel to a 100-mL volumetric flask, and dilute to volume with the washings obtained by rinsing the beaker, electrodes and stirrer several times with small quantities of distilled water. Stopper the flask and invert several times to mix the contents.

To determine the different forms of N in the hydrolysate, after thorough mixing, usually a 5- to 10-mL sample is pipetted into a 50- or 100-mL distillation flask and the flask is connected to a steam distillation apparatus. It is necessary to use pipettes with wide tips that permit rapid delivery to avoid sampling errors. The form of N under analysis is determined from the NH_3-N liberated by steam distillation for 2 to 4 min.

39–4.3.4 Total Hydrolyzable Nitrogen

Place 5 mL of the neutralized hydrolysate in a 50-mL distillation flask, add 0.5 g of K_2SO_4-catalyst mixture and 2 mL of concentrated H_2SO_4, and heat the flask cautiously on a micro-Kjeldahl digestion unit until the water is removed and frothing ceases. Increase the heat until the mixture clears, and complete the digestion by boiling gently for 1 h.

After digestion, allow the flask to cool, and add about 10 mL of water (slowly and with shaking). Cool the flask under a cold-water tap, and place it in a beaker containing crushed ice. Add 5 mL of H_3BO_3 indicator solution to a 50-mL Erlenmeyer flask that is marked to indicate a volume of 35 mL, and place the flask under the condenser of the steam distillation apparatus so that the tip of the condenser is about 4 cm above the surface of the H_3BO_3. Connect the cooled distillation flask to the distillation ap-

paratus, place 10 mL of 10 *M* NaOH in the entry funnel, and run the alkali slowly into the distillation flask. When about 0.5 mL of alkali remains in the funnel, rinse the funnel rapidly with about 5 mL of water, and allow about 2 mL to run into the distillation flask before sealing the funnel. Commence steam distillation and stop when the distillate reaches the 35-mL mark on the receiver flask (distillation time about 4 min). Rinse the condenser, and determine the NH_4^+-N in the distillate by titration with 0.0025 *M* H_2SO_4 from a microburette (1 mL = 70 μg NH_4^+-N). The color change at the endpoint is from green to a faint, permanent pink.

39–4.3.5 Acid-insoluble Nitrogen

This form of N is the difference between total soil N (Bremner & Mulvaney, 1982) and total hydrolyzable N (section 39–4.3.4). It can also be determined directly by acid digestion of the soil residue remaining after hydrolysis (Cheng & Kurtz, 1963).

39–4.3.6 Amino Acid-Nitrogen

Place 5 mL of the hydrolysate (section 39–4.3.3) in a 50-mL distillation flask, add 1 mL of 0.5 *M* NaOH, and heat the flask in boiling water until the volume of the sample is reduced to 2 to 3 mL (approximately 20 min). Allow the flask to cool, add 500 mg of citric acid and 100 mg of ninhydrin, and place the flask in a vigorously boiling water bath, so that its bulb is completely immersed in boiling water. After about 1 min, swirl the flask for a few seconds without removing it from the bath, and allow it to remain in the bath for an additional 9 min. Then cool the flask, add 10 mL of phosphate-borate buffer and 1 mL of 5 *M* NaOH, and connect the flask to the steam distillation apparatus. Determine the amount of NH_3-N liberated by steam distillation as in section 39–4.3.4 (distillation period about 4 min).

39–4.3.7 Ammonia-Nitrogen

Place 10 mL of the hydrolysate (section 39–4.3.3) in a 50- or 100-mL distillation flask, add 0.07 ± 0.01 g of MgO, and connect the flask to the steam distillation apparatus. Determine the amount of NH_3-N liberated by steam distillation as in section 39–4.3.4, but collect the distillate in a 50-mL Erlenmeyer flask that contains 5 mL of H_3BO_3-indicator solution and marked to indicate a volume of 20 mL. Discontinue distillation when the distillate reaches the 20-mL mark (distillation period about 2 min).

39–4.3.8 (Ammonia + Amino Sugar)-Nitrogen

Place 10 mL of the hydrolysate (section 39–4.3.3) in a 100-mL distillation flask, add 10 mL of phosphate-borate buffer, and connect the flask to the distillation apparatus. Determine the amount of NH_3-N liberated by steam distillation as described in section 39–4.3.4 (distillation period about 4 min).

39–4.3.9 Amino Sugar-Nitrogen

This form of N is taken as the difference between the amounts of N recovered in the preceding two sections.

39–4.3.10 (Serine + Threonine)-Nitrogen

Proceed as described in section 39–4.3.8, but after removal of (NH_3 + amino sugar)-N by steam distillation with phosphate-borate buffer, detach the flask from the distillation apparatus, and rinse the steam inlet tube with 3 to 5 mL of water. Collect the rinse water in the distillation flask, and cool the flask under a cold water tap. Add 2 mL of periodic acid solution, swirl the flask for about 30 s, add 2 mL of sodium arsenite solution, and connect the flask to the distillation apparatus. Determine the amount of NH_3-N liberated by steam distillation as described in section 39–4.3.4 (period of distillation about 4 min).

39–4.3.11 Comments

One of the main advantages of the described hydrolysis method is that the N in the different fractions is measured as NH_4^+-N, and this can be readily converted to N_2 for isotope-ratio analysis. Total N in a given sample may be insufficient for mass spectrometer analysis, and duplicate analyses may have to be combined for ^{15}N determinations. If the ^{15}N percentage is relatively high, it may be diluted with a measured amount of unlabeled N to provide sufficient total N for mass spectrometer requirements (Hauck, 1982). The use of ^{15}N-labeled soils provides a means of tracing the movement of added N into the various organic fractions and determination of the rate at which this occurs if a proper time sequence is employed. Allen et al. (1973) present some typical data that can be obtained by this means.

The recommended hydrolysis procedure can be modified in several ways, but the more extensive discussion of the procedure by Bremner (1965) and Stevenson (1982b) should be consulted before doing so. It should also be pointed out that the hydrolysis method presented here causes greater decomposition of amino sugars than more conventional methods. The correction factor for hydrolysis losses of amino sugars is about 1.4 (Bremner, 1965).

39–5 CONCLUSIONS

During the past 50 yr, the use of isotopes in the study of soil organic matter dynamics has led to a tremendous increase in knowledge of the system that would not have been possible otherwise. Numerous methods for the use of isotopes have been developed over the years with specific objectives in mind and equipment available at the time. This chapter outlines basic methods currently applicable to organic matter studies, recognizing that improvements and modifications are constantly being made.

The References section provides detailed information on techniques that have been developed.

REFERENCES

Allen, A.L., F.J. Stevenson, and L.T. Kurtz. 1973. Chemical distribution of residual fertilizer nitrogen in soil as revealed by nitrogen-15 studies. J. Environ. Qual. 2:120–124.

Allison, F.E. 1973. Soil organic matter and its role in crop production. Elsevier Scientific Publ. Co., New York.

Andersen, A., G. Nielsen, and H. Sorensen. 1961. Growth chamber for labeling plant material uniformly with radiocarbon. Physiol. Plant. 14:378–383.

Anderson, J.P.E. 1982. Soil respiration. p. 831–871. *In* A.L. Page et al. (ed.) Methods of soil analysis. Part 2. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

Andreux, F., C. Cerri, P.B. Vose, and V.A. Vitorello. 1990. Potential of stable isotope, ^{15}N and ^{13}C, methods for determining input and turnover in soils. p. 259–275. *In* A.F. Harrison et al. (ed.) Nutrient cycling in terrestrial ecosystems. Elsevier Applied Sci., New York.

Atlas, R.M., and R. Bartha. 1972. Degradation and mineralization of petroleum by two bacteria isolated from coastal waters. Biotechnol. Bioeng. 14:297–308.

Balesdent, J., A. Mariotti, and D. Boisgontier. 1990. Effect of tillage on soil organic carbon mineralization estimated from ^{13}C abundance in maize fields. J. Soil Sci. 41:587–596.

Balesdent, J., A. Mariotti, and B. Guillet. 1987. Natural ^{13}C abundance as a tracer for studies of soil organic matter dynamics. Soil Biol. Biochem. 19:25–30.

Balesdent, J., G.H. Wagner, and A. Mariotti. 1988. Soil organic matter turnover in long-term field experiments as revealed by carbon-13 natural abundance. Soil Sci. Soc. Am. J. 52:118–124.

Bartha, R., and D. Pramer. 1965. Features of a flask and method for measuring the persistence and biological effects of pesticides in soil. Soil Sci. 100:68–70.

Boutton, T.W. 1991a. Stable carbon isotope ratios of natural materials: I. Sample preparation and mass spectrometric analysis. p. 155–171. *In* D.C. Coleman and B. Fry (ed.) Carbon isotope techniques. Academic Press, New York.

Boutton, T.W. 1991b. Stable carbon isotope ratios of natural materials: II. Atmospheric, terrestrial, marine, and freshwater environments. p. 173–185. *In* D.C. Coleman and B. Fry (ed.) Carbon isotope techniques. Academic Press, New York.

Boutton, T.W., A.T. Harrison, and B.N. Smith. 1980. Distribution of biomass of species differing in photosynthetic pathway along an altitudinal transect in southeastern Wyoming grassland. Oecologia 45:287–298.

Boutton, T.W., W.W. Wong, D.L. Hachey, L.S. Lee, M.P. Cabrera, and P.D. Klein. 1983. Comparison of quartz and Pyrex tubes for combustion of organic samples for stable carbon isotope analysis. Anal. Chem. 55:1832–1833.

Bremner, J.M. 1965. Organic forms of nitrogen. p. 1238–1255. *In* C.A. Black et al. (ed.) Methods of soil analysis. Part 2. Agron. Monogr. 9. ASA, Madison, WI.

Bremner, J.M., and C.S. Mulvaney. 1982. Nitrogen-total. p. 595–624. *In* A.L. Page et al. (ed.) Methods of soil analysis. Part 2. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

Broadbent, F.E., and T. Nakashima. 1967. Reversion of fertilizer nitrogen in soils. Soil Sci. Soc. Am. Proc. 31:648–652.

Broadbent, F.E., and T. Nakashima. 1974. Mineralization of carbon and nitrogen in soil amended with carbon-13 and nitrogen-15 labeled plant material. Soil Sci. Soc. Am. Proc. 38:313–315.

Cerri, C.C., B. Volkoff, and F. Andreux. 1991. Nature and behavior of organic matter in soils under natural forest, and after deforestation, burning and cultivation, near Manaus. Forest Ecol. Manage. 38:247–257.

Cheng, H.H., and L.T. Kurtz. 1963. Chemical distribution of added nitrogen in soils. Soil Sci. Soc. Am. Proc. 27:312–316.

Cheshire, M.V., and B.S. Griffiths. 1989. The influence of earthworms and cranefly larvae on the decomposition of uniformly ^{14}C labeled plant material in soil. J. Soil Sci. 40:117–124.

Chichester, F.W. 1970. Transformation of fertilizer nitrogen in soil: II. Total and ^{15}N labeled nitrogen of soil organomineral sedimentation fractions. Plant Soil 33:437–456.

Chichester, F.W., J.O. Legg, and G. Stanford. 1975. Relative mineralization rates of indigenous and recently incorporated ^{15}N-labeled nitrogen. Soil Sci. 120:455–460.

Coleman, D.C., and F.T. Corbin. 1991. Introduction and ordinary counting as currently used. p. 3–9. *In* D.C. Coleman and B. Fry (ed.) Carbon isotope techniques. Academic Press, New York.

Corbin, F.T., and B.A. Swisher. 1986. Radioisotope techniques. p. 265–276. *In* N.D. Camper (ed.) Research methods in weed science. Southern Weed Sci. Soc., Champaign, IL.

Craig, H. 1957. Isotopic standards for carbon and oxygen and correction factors for mass spectrometric analysis of carbon dioxide. Geochim. Cosmochim. Acta 12:133–149.

Crawford, D.L. 1978. Lignocellulose decomposition by selected *Streptomyces* strains. Appl. Environ. Microbiol. 35:1041–1045.

Crawford, D.L., and R.L. Crawford. 1976. Microbial degradation of lignocellulose: The lignin component. Appl. Environ. Microbiol. 31:714–717.

Crawford, D.L., R.L. Crawford, and A.L. Pometto. 1977. Preparation of specifically labeled ^{14}C-(lignin)- and ^{14}C-(cellulose)-lignocelluloses and their decomposition by the microflora of soil. Appl. Environ. Microbiol. 33:1247–1251.

Deines, P. 1970. Mass spectrometer correction factors for the determination of small isotopic composition variations of carbon and oxygen. Int. J. Mass Spectrom. Ion Phys. 4:283–295.

Des Marais, D., and J.M. Hayes. 1976. Tube cracker for opening glass-sealed ampoules under vacuum. Anal. Chem. 48:1651–1652.

Dzurec, R.S., T.W. Boutton, M.M. Caldwell, and B.N. Smith. 1985. Carbon isotope ratios of soil organic matter and their use in assessing community composition changes in Curlew Valley, Utah. Oecologia 66:17–24.

Engel, M.H., and R.J. Maynard. 1989. Preparation of organic matter for stable carbon isotope analysis by sealed tube combustion: A cautionary note. Anal. Chem. 61:1996–1998.

Frazer, J.W., and R. Crawford. 1963. Modifications in the simultaneous determination of carbon, hydrogen, and nitrogen. Mikrochim. Acta 3:561–566.

Fried, M., and L.A. Dean. 1952. A concept concerning the measurement of available soil nutrients. Soil Sci. 73:263–271.

Gauch, H.G. 1972. Inorganic plant nutrition. Dowden, Hutchinson & Ross, Stroudsburg, PA.

Goh, K.M. 1991. Bomb carbon. p. 147–151. *In* D.C. Coleman and B. Fry (ed.) Carbon isotope techniques. Academic Press, New York.

Gonfiantini, R. 1981. The δ-notation and the mass spectrometric measurement techniques. p. 35–84. *In* J.R. Gat and R. Gonfiantini (ed.) Stable isotope hydrology. IAEA, Vienna, Austria.

Haider, K., and J.P. Martin. 1975. Decomposition of specifically carbon-14 labeled benzoic and cinnamic acid derivatives in soil. Soil Sci. Soc. Am. Proc. 39:657–662.

Haider, K., J.P. Martin, and E. Rietz. 1977. Decomposition in soil of ^{14}C-labeled coumaryl alcohols; free and linked into dehydropolymer and plant lignins and model humic acids. Soil Sci. Soc. Am. J. 41:556–562.

Harris, D., and E.A. Paul. 1991. Techniques for examining the carbon relationships of plant-microbial symbioses. p. 39–52. *In* D. C. Coleman and B. Fry (ed.) Carbon isotope techniques. Academic Press, New York.

Harrison, A.F., D.D. Harkness, and P.J. Bacon. 1990. The use of bomb-^{14}C for studying organic matter and N and P dynamics in a woodland soil. p. 246–258. *In* A.F. Harrison et al. (ed.) Nutrient cycling in terrestrial ecosystems. Elsevier Applied Sci., New York.

Hauck, R.D. 1982. Nitrogen-isotope ratio analysis. p. 735–779. *In* A.L. Page et al. (ed.) Methods of soil analysis. Part 2. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

Hayes, J.M. 1983. Practice and principles of isotopic measurements in organic geochemistry. p. 5.1–5.31. *In* W.G. Meinschein (ed.) Organic geochemistry of contemporaneous and ancient sediments. Soc. for Econ. Paleontologists and Mineralogists, Bloomington, IN.

Hurst, H.M., and G.H. Wagner. 1969. Decomposition of ^{14}C-labeled cell wall and cytoplasmic fractions from hyaline and melanic fungi. Soil Sci. Soc. Am. Proc. 33:707–711.

Insam, H., M.M. Ding, and A. Mariotti. 1991. Utilization of a ^{13}C-enriched tracer for carbon flux studies in a tropical *Eucalyptus exserta* forest. p. 515–519. *In* Stable isotopes in plant nutrition, soil fertility, and environmental studies. IAEA, Vienna, Austria.

Jackson, M.L. 1969. Soil chemical analysis—Advanced course. 2nd ed. 11th printing. Published by the author, Madison, WI.

Jansson, S.L. 1958. Tracer studies on nitrogen transformations in soil. Annu. Roy. Agric. Coll. Sweden 24:101–361.

Jansson, S.L., and J. Persson. 1982. Mineralization and immobilization of soil nitrogen. p. 229–252. *In* F.J. Stevenson (ed.) Nitrogen in agricultural soils. Agron. Monogr. 22. ASA, CSSA, and SSSA, Madison, WI.

Jenkinson, D.S. 1960. The production of ryegrass labeled with carbon-14. Plant Soil 13:279–290.

Jenkinson, D.S. 1971. Studies on the decomposition of ^{14}C-labeled organic matter in soil. Soil Sci. 111:64–70.

Jenkinson, D.S., and J.N. Ladd. 1981. Microbial biomass in soil: Measurement and turnover. p. 415–471. *In* E.A. Paul and J.N. Ladd (ed.) Soil biochemistry. Vol. 5. Marcel Dekker, New York.

Jenkinson, D.S., and D.S. Powlson. 1976. The effects of biocidal treatments on metabolism in soil. V: A method for measuring biomass. Soil Biol. Biochem. 8:209–213.

Jenkinson, D.S., and J.H. Rayner. 1977. The turnover of soil organic matter in some Rothamsted classical experiments. Soil Sci. 123:298–305.

Kassim, G., J.P. Martin, and K. Haider. 1981. Incorporation of a wide variety of organic substrate carbons into soil biomass as estimated by the fumigation procedure. Soil Sci. Soc. Am. J. 45:1106–1112.

Kassim, G., D.E. Stott, J.P. Martin, and K. Haider. 1982. Stabilization and incorporation into biomass of phenolic and benzenoid carbons during biodegradation in soil. Soil Sci. Soc. Am. J. 46:305–309.

Kearney, P.C., and A. Kontson. 1976. A simple system to simultaneously measure volatilization and metabolism of pesticides from soils. J. Agric. Food Chem. 24:424–426.

Keeney, D.R., and D.W. Nelson. 1982. Nitrogen-inorganic forms. p. 643–698. *In* A.L. Page et al. (ed.) Methods of soil analysis. Part 2. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

Kelley, K.R., and F.J. Stevenson. 1985. Characterization and extractability of immobilized ^{15}N from the soil microbial biomass. Soil Biol. Biochem. 17:517–523.

Kemper, W.D., and R.C. Rosenau. 1986. Aggregate stability and size distribution. p. 425–442. *In* A. Klute (ed.) Methods of soil analysis. Part 1. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

Ladd, J.N., and M.A. Amato. 1986. The fate of nitrogen from legume and fertilizer sources in soils successively cropped with wheat under field conditions. Soil Biol. Biochem. 18:417–425.

Ladd, J.N., and J.K. Martin. 1984. Soil organic matter studies. p. 67–98. *In* M.F. L'Annunziata and J.O. Legg (ed.) Isotopes and radiation in agricultural sciences. Vol. 1. Academic Press, London.

Ladd, J.N., J.M. Oades, and M. Amato. 1981. Distribution and recovery of nitrogen from legume residues decomposing in soils sown to wheat in the field. Soil Biol. Biochem. 13:251–256.

L'Annunziata, M.F. 1979. Radiotracers in agricultural chemistry. Academic Press, New York.

L'Annunziata, M.F. 1984. The detection and measurement of radionuclides. p. 141–231. *In* M.F. L'Annunziata and J.O. Legg (ed.) Isotopes and radiation in agricultural sciences. Vol. 1. Academic Press, London.

Lavy, T.L. 1975. Effects of soil pH and moisture on the direct radioassay of herbicides in soil. Weed Sci. 23:49–52.

Le Feuvre, R.P., and R.J. Jones. 1988. Static combustion of biological samples sealed in glass tubes as a preparation for $\delta^{13}C$ determination. Analyst 113:817–823.

Legg, J.O., F.W. Chichester, G. Stanford, and W.H. DeMar. 1971. Incorporation of ^{15}N-tagged mineral nitrogen into stable forms of soil organic nitrogen. Soil Sci. Soc. Am. Proc. 35:273–276.

Levin, I., B. Kromer, D. Wagenback, and K.O. Munnich. 1987. Carbon isotope measurements of atmospheric CO_2 at a coastal station in Antarctica. Tellus 39B:89–95.

Loos, M.A., A. Kontson, and P.C. Kearney. 1980. Inexpensive soil flask for ^{14}C-pesticide degradation studies. Soil Biol. Biochem. 12:583–585.

Martin, A., A. Mariotti, J. Balesdent, P. Lavelle, and R. Vuattoux. 1990. Estimate of organic matter turnover rate in a savanna soil by ^{13}C natural abundance measurements. Soil Biol. Biochem. 22:517–523.

Martin, J.P., and K. Haider. 1979. Biodegradation of ^{14}C-labeled model and cornstalk lignins, phenols, model phenolase humic polymers, and fungal melanins as influenced by a readily available carbon source and soil. Appl. Environ. Microbiol. 38:283–289.

Martin, J.P., K. Haider, and D.C. Wolf. 1972. Synthesis of phenols and phenolic polymers by *Hendersonula toruloidea* in relation to humic acid formation. Soil Sci. Soc. Am. Proc. 36:311–315.

Martin, J.P., H. Zunino, P. Peirano, M. Caiozzi, and K. Haider. 1982. Decomposition of ^{14}C-labeled lignins, model humic acid polymers, and fungal melanins in allophanic soils. Soil Biol. Biochem. 14:289–293.

Marvel, J.T., B.B. Brightwell, J.M. Malik, M.L. Sutherland, and M.L. Rueppel. 1978. A simple apparatus and quantitative method for determining the persistence of pesticides in soil. J. Agric. Food Chem. 26:1116–1120.

Mayaudon, J. 1971. Use of radiorespirometry in soil microbiology and biochemistry. p. 202–256. *In* A.D. McLaren and J. Skujins (ed.) Soil biochemistry. Vol. 2. Marcel Dekker, New York.

Melillo, J.M., J.D. Aber, A.E. Linkins, A. Ricca, B. Fry, and K.J. Nadelhoffer. 1989. Carbon and nitrogen dynamics along the decay continuum: Plant litter to soil organic matter. p. 53–62. *In* M. Clarholm and L. Bergstrom (ed.) Ecology of arable land. Kluwer Acad. Publ., Dordrecht, Netherlands.

Mook, W.G., and P.M. Grootes. 1973. The measuring procedure and corrections for the high precision mass spectrometric analysis of isotopic abundance ratios, especially referring to carbon, oxygen, and nitrogen. Int. J. Mass Spectrom. Ion Phys. 12:273–298.

Mulvaney, R.L. 1993. Mass spectrometry. p. 11–57. *In* R. Knowles and T.H. Blackburn (ed.) Nitrogen isotope techniques. Academic Press, San Diego.

Myrold, D.D., and J.M. Tiedje. 1986. Simultaneous estimation of several nitrogen cycle rates using ^{15}N: Theory and application. Soil Biol. Biochem. 18:559–568.

Nadelhoffer, K.J., and B. Fry. 1988. Controls on natural nitrogen-15 and carbon-13 abundances in forest soil organic matter. Soil Sci. Soc. Am. J. 52:1633–1640.

Nakas, J.P., and D.A. Klein. 1979. Decomposition of microbial cell components in a semi-arid grassland soil. Appl. Environ. Microbiol. 38:454–460.

Nelson, D.W., and L.E. Sommers. 1982. Total carbon, organic carbon, and organic matter. p. 539–580. *In* A.L. Page et al. (ed.) Methods of soil analysis. Part 2. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

Oades, J.M., and G.H. Wagner. 1971. Biosynthesis of sugars in soils incubated with ^{14}C-glucose and ^{14}C-dextran. Soil Sci. Soc. Am. Proc. 35:914–917.

O'Leary, M.H. 1988. Carbon isotopes in photosynthesis. BioScience 38:328–336.

Osmond, C.B., K. Winter, and H. Ziegler. 1982. Functional significance of different pathways of CO_2 fixation in photosynthesis. p. 479–547. *In* O.L. Lange et al. (ed.) Plant physiological ecology II: Water relations and carbon assimilation. Springer-Verlag, Berlin.

Paul, E.A., and F.E. Clark. 1989. Soil microbiology and biochemistry. Academic Press, New York.

Paul, E.A., and J.A. van Veen. 1978. The use of tracers to determine the dynamic nature of organic matter. p. 61–102. Trans. 11th Int. Congr. Soil Sci. Vol. 3. Edmonton, Canada.

Post, W.M., W.R. Emanuel, P.J. Zinke, and A.G. Stangenberger. 1982. Soil carbon pools and world life zones. Nature (London) 298:156–159.

Post, W.M., J. Pastor, P.J. Zinke, and A.G. Stangenberger. 1985. Global patterns of soil nitrogen storage. Nature (London) 317:613–616.

Prentice, K.C., and I.Y. Fung. 1990. The sensitivity of terrestrial carbon storage to climate change. Nature (London) 346:48–51.

Reyes, V.G., and J.M. Tiedje. 1973. Metabolism of ^{14}C uniformly labeled organic material by woodlice (Isopoda: Oniscoidea) and soil microorganisms. Soil Biol. Biochem. 5: 603–611.

Russell, E.W. 1961. Soil conditions and plant growth. John Wiley and Sons, New York.

Santrock, J., S. Studley, and J. Hayes. 1985. Isotopic analyses based on the mass spectrum of carbon dioxide. Anal. Chem. 57:1444–1448.

Scharpenseel, H.W., and H.U. Neue. 1984. Use of isotopes in studying the dynamics of organic matter in soils. p. 273–310. *In* Organic matter and rice. Int. Rice Res. Inst., Manila, Philippines.

Schlesinger, W.H. 1990. Evidence from chronosequence studies for a low carbon storage potential of soils. Nature (London) 348:232–234.

Schnitzer, M. 1982. Organic matter characterization. p. 581–594. *In* A.L. Page et al. (ed.) Methods of soil analysis. Part 2. 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

Scott, H.D., and R.E. Phillips. 1972. Diffusion of selected herbicides in soil. Soil Sci. Soc. Am. Proc. 36:714–719.

Scully, N.J., W. Chorney, G. Kostal, R. Watanabe, J. Skok, and J.W. Glattfeld. 1956. Biosynthesis in ^{14}C-labeled plants: Their use in agricultural and biological research. Vol. 12. p. 377–385. *In* Proc. Int. Conf. Peaceful Uses of Atomic Energy, Geneva, Switzerland, 8–20 Aug. 1955. United Nations, New York.

Sissons, C.H. 1976. Improved technique for accurate and convenient assay of biological reactions liberating $^{14}CO_2$. Anal. Biochem. 70:454–462.

Skjemstad, J.O., R.P. Le Feuvre, and R.E. Prebble. 1990. Turnover of soil organic matter under pasture as determined by ^{13}C natural abundance. Aust. J. Soil Res. 28:267–276.

Smith, B.N., and S. Epstein. 1971. Two categories of $^{13}C/^{12}C$ ratios for higher plants. Plant Physiol. 47:380–384.

Smith, J.H., F.E. Allison, and J.F. Mullins. 1962. Design and operation of a carbon-14 biosynthesis chamber. USDA-ARS Misc. Publ. 911. U.S. Gov. Print. Office, Washington, DC.

Sofer, Z. 1980. Preparation of carbon dioxide for stable carbon isotope analysis of petroleum fractions. Anal. Chem. 52:1389–1391.

Stevenson, F.J. 1965. Gross chemical fractionation of organic matter. p. 1409–1421. *In* C.A. Black et al. (ed.) Methods of soil analysis. Part 2. Agron. Monogr. 9. ASA, Madison, WI.

Stevenson, F.J. 1982a. Humus chemistry, genesis, composition, reactions. John Wiley and Sons, New York.

Stevenson, F.J. 1982b. Nitrogen—organic forms. p. 625–641. *In* A.L. Page et al. (ed.) Methods of soil analysis. Part 2, 2nd ed. Agron. Monogr. 9. ASA and SSSA, Madison, WI.

Stevenson, F.J. 1986. Cycles of soil: Carbon, nitrogen, phosphorus, sulfur, micronutrients. John Wiley and Sons, New York.

Stevenson, F.J., and E.T. Elliott. 1989. Methodologies for assessing the quantity and quality of soil organic matter. p. 173–199. *In* D.C. Coleman et al. (ed.) Dynamics of soil organic matter in tropical ecosystems. NifTAL Project, Dep. Agronomy and Soil Science, Univ. of Hawaii, Honolulu.

Stewart, B.A., L.K. Porter, and D.D. Johnson. 1963. Immobilization and mineralization of nitrogen in several organic fractions of soil. Soil Sci. Soc. Am. Proc. 27:302–304.

Stotzky, G. 1965. Microbial respiration. p. 1550–1572. *In* C.A. Black et al. (ed.) Methods of soil analysis. Part 2. Agron. Monogr. 9. ASA, Madison, WI.

Stotzky, G., M.W. Broder, J.D. Doyle, and R.A. Jones. 1993. Selected methods for the detection and assessment of ecological effects resulting from the release of genetically engineered microorganisms to the terrestrial environment. *In* S.L. Neidleman and A.I. Laskin (ed.) Adv. Appl. Microbiol. 38:1–93.

Stout, J.D., K.M. Goh, and T.A. Rafter. 1981. Chemistry and turnover of naturally occurring resistant organic compounds in soil. p. 1–73. *In* E.A. Paul and J.N. Ladd (ed.) Soil biochemistry. Vol. 5. Marcel Dekker, New York.

Swerhone, G.D.W., K.A. Hobson, C. van Kessel, and T.W. Boutton. 1991. An economical method for the preparation of plant and animal tissue for $\delta^{13}C$ analysis. Commun. Soil Sci. Plant Anal. 22:177–190.

Teeri, J.A., and L.G. Stowe. 1976. Climatic patterns and the distribution of C_4 grasses in North America. Oecologia 23:1–12.

Tiessen, H., and J.W.B. Stewart. 1983. Particle size fractions and their use in studies of soil organic matter: II. Cultivation effects on organic matter composition in size fractions. Soil Sci. Soc. Am. J. 47:509–514.

Verma, L., J.P. Martin, and K. Haider. 1975. Decomposition of carbon-14-labeled proteins, peptides, and amino acids; free and complexed with humic polymers. Soil Sci. Soc. Am. Proc. 39:279–284.

Vitorello, V.A., C.C. Cerri, F. Andreux, C. Feller, and R.L. Victoria. 1989. Organic matter and natural carbon-13 distribution in forested and cultivated Oxisols. Soil Sci. Soc. Am. J. 53:773–778.

Voroney, R.P., J.P. Winter, and E.G. Gregorich. 1991. Microbe/plant/soil interactions. p. 77–99. *In* D.C. Coleman and B. Fry (ed.) Carbon isotope techniques. Academic Press, New York.

Wagner, G.H. 1975. Microbial growth and carbon turnover. p. 269–305. *In* E.A. Paul and A.D. McLaren (ed.) Soil biochemistry. Vol. 3. Marcel Dekker, New York.

Wagner, G.H., and K.S. Chahal. 1966. Decomposition of carbon-14 labeled atrazine in soil samples from Sanborn Field. Soil Sci. Soc. Am. Proc. 30:752–754.

Wagner, G.H., and A.M. Krzywicka. 1975. Decomposition of algal tissues in soil. p. 202–207. *In* G. Kilbertus et al. (ed.) Biodegradation et humification. Pierron Publ., Sarreguemines, France.

Warembourg, F.R., and J. Kummerow. 1991. Photosynthesis/translocation studies in terrestrial ecosystems. p. 11–37. *In* D.C. Coleman and B. Fry (ed.) Carbon isotope techniques. Academic Press, New York.

Wolf, D.C., and J.O. Legg. 1984. Soil microbiology. p. 99–139. *In* M.F. L'Annunziata and J.O. Legg (ed.) Isotopes and radiation in agricultural sciences. Vol. 1. Academic Press, London.

Zunino, H., F. Borie, S. Aguilera, J.P. Martin, and K. Haider. 1982. Decomposition of ^{14}C-labeled glucose, plant and microbial products and phenols in volcanic ash-derived soils of Chile. Soil Biol. Biochem. 14:37–43.