Chapter 3

Characterization of Soil Organic Carbon Relative to Its Stability and Turnover

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I. INTRODUCTION

A wide variety of techniques have been applied to the measurement and characterization of soil organic matter (SOM) in relation to its dynamics under various management conditions. The selection of methods

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to describe SOM dynamics depends upon the purpose of the study, be it for chemical characterization and identification of specific SOM components, the description of SOM pools important in the cycling and release of plant nutrients, or the quantification of ecosystem carbon (C) budgets. Classical approaches have combined chemical extractions with identification of specific chemical compounds. Functional approaches have attempted to provide a description of SOM pool dynamics by incorporating radio- and stable isotopes as tracers or using ^{14}C dating techniques to identify specific fractions that are biologically active. Stevenson and Elliott[1] stressed that methods used to evaluate SOM dynamics should be related to fractions or pools that have biological significance if they are to relate to the potential for soils to provide nutrients to plants. In the following we discuss methods that permit functional descriptions of SOM transformations relative to ecosystem functioning, biodegradation, soil fertility, and global change.

II. TOTAL SOIL ORGANIC MATTER: ORGANIC CARBON

Estimates of SOM are derived primarily from the determination of total organic C since it comprises 48 to 58% of SOM mass.[2] The most commonly used analytical procedures involve dry combustion or wet digestion. In dry combustion, total C is determined by burning soil in a stream of pure O_2 in a resistance or induction furnace; CO_2 is determined by titration or thermal conductivity. In wet combustion, soil is digested in the strong oxidant $K_2Cr_2O_7$ and a 3:1 mixture of H_2SO_4 and H_3PO_4. Oxidizable C is determined by either titration of excess $K_2Cr_2O_7$ or the measurement of liberated CO_2 trapped in 1 *N* NaOH, which is then titrated with HCl after addition of $BaCl_2$.[3] For a complete description of the materials and methods involved in total C determination see the review of Nelson and Sommers.[2]

Recent improvements in automated instruments such as the Carlo Erba CHN* (Carlo Erba Strumentazione, Milan), LECO CHN-600 (Laboratory Equipment Corp., St. Joseph, MO), or Perkin-Elmer total C and N analyzers have increased the precision and accuracy of determining total soil C. Although the initial cost of this type of equipment is high, large numbers of samples can be run in a shorter period of time, compared to wet oxidation.[4] Sheldrick,[5] in comparing the LECO CHN-600 to wet oxidation, concluded that the automated method was preferable because it provided more reliable and precise data and was more efficient since both total C and N could be determined faster than C alone in a single analysis. The small sample size often associated with automated techniques requires very fine grinding of soil. Fine grinding of soil to particle sizes ranging to below 0.08 mm has been reported using hammer-, ball-, or roller-mill grinders.[6-8] Multisample conveyor-belt grinders that can be built with normal laboratory equipment and supplies have been described by Smith and Um[9] and Kelly.[10]

III. ISOLATION AND FRACTIONATION OF SOIL ORGANIC MATTER

A. CHEMICAL EXTRACTION AND PURIFICATION

Classical chemical fractionation of SOM yields three major fractions: humic acids (HA), fulvic acids (FA), and humin.[11] This technique is based upon differences in solubilities of humic substances in alkaline and acid solutions. The technique involves extraction with an alkaline reagent, usually NaOH or $Na_4P_2O_7$, separation of the soluble extract from the soil residue, following acidification of the extract to pH 2 with HCl (Figure 1A). FAs are soluble in both alkali and acid, HAs are soluble in alkali but precipitated by acid, and humin is insoluble in both alkali and acid. The fractions should not be considered distinct or discrete compounds since each can be further purified to reduce heterogeneity.[12,13] Common techniques used to purify humic extracts include electrophoresis, electro-ultra filtration, ion exchange, gel chromatography, salting out, changes in pH, and further use of differences in solubility. Humic substances differ in molecular weight, elemental composition, acidity, and cation exchange capacity (Figure 1B).

Fulvic extracts are typically composed of a variety of phenolic and benzene carboxylic acids that are held together by hydrogen bonds to form stable polymeric structures.[14] These are associated with polysaccharides that are easily separated by adsorption on charcoal or gel and resin chromatography. The low molecular weight FAs have higher oxygen but lower C contents than HAs and contain more acidic functional groups, particularly COOH.[15] The HA fraction consists of hydroxyphenols, hydroxybenzoic acids, and other aromatic structures with linked peptides, amino compounds, and fatty acids.

* Trade names and company names are included for the benefit of the reader and do not imply any endorsement or preferential treatment of the product listed.

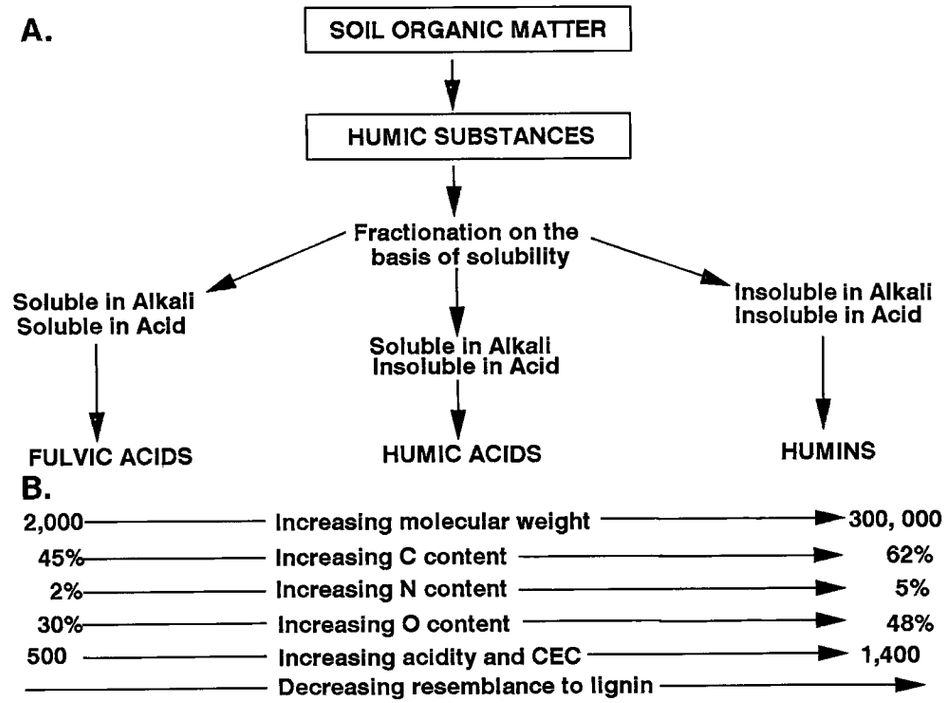

Figure 1 (A) Classical fractionation scheme of SOM and (B) characteristic properties of humic substances.[1,11,13]

Humic extracts are generally contaminated with silicates that can be removed with HF. Proteins and carbohydrates can be removed using 6 *M* HCl.

Soil humins are considered to be nonextractable humic type polymers that form strong associations with the mineral fraction and are not as easily separated by the usual alkaline reagents.[14] Hatcher et al.[16] suggested that the humin fraction consisted of highly condensed HAs, fungal melanins, and paraffinic structures. Almendros and Gonzalez-Vila[17] found a high proportion of polymethylene compounds (fatty acids) that seem to be inherited from the waxes of higher plants and further suggested that, during biodegradation, the lipid polymers are altered and incorporated into the humin fraction. Although HAs and humins constitute the majority of organic C in a system, they contribute only a small portion to the annual cycling of soil C because of their extremely slow turnover rate. The aromatic HAs acids are considered to be very stable in soil,[18] corresponding to the chemical stabilized organic matter postulated by Jenkinson and Rayner[19] and van Veen and Paul.[20] FAs have turnover times of hundreds of years, whereas those HAs and humins approach several thousand years.[21]

An alternative to NaOH or $Na_4P_2O_7$ extraction involves acid hydrolysis. This simple rapid technique provides an acid-soluble fraction ranging from 25 to 50% of the soil C and a nonhydrolyzable fraction constituting 50 to 75% of the C. Experiments with ^{14}C involving both C dating and enriched samples show that there are some artifacts, e.g., modern lignin ends up in the non-hydrolyzable plant fraction. However, there still is usually a difference of 1000 years in the C age of the soluble and much older acid-soluble fractions giving an estimate of the size and tracer age of the resistant old fractions.

B. HUMUS FORMATION

The prevailing theory of humus formation is based upon a multistage process involving (1) decomposition of plant material to simple C compounds; (2) assimilation and repeated cycling of C through the microbial biomass with formation of new cells; and (3) concurrent polymerization of microbial synthesized polyphenols (quinones) and alteration of plant-derived lignin to form high molecular weight polymers (Figure 2).[15,22,23]

Plant material is the primary source of material for SOM formation. Decomposition products are incorporated into various SOM fractions. In general, water-soluble C compounds (simple sugars, proteins) degrade first, followed by structural polysaccharides (cellulose and hemicelluloses), and then

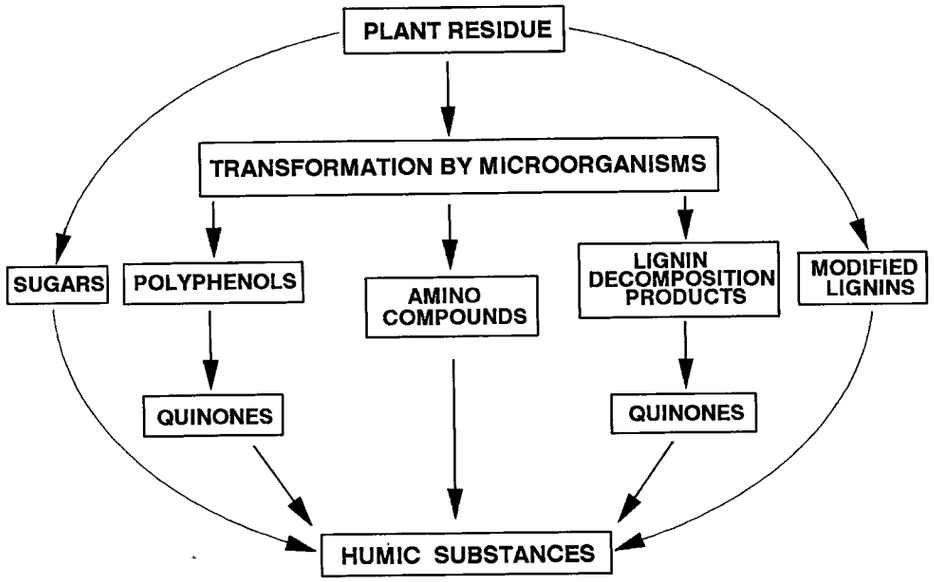

Figure 2 Transformation processes during the formation of humic substances.[11,13]

lignin, which decomposes at a much slower rate. Initially, between 40 and 60% of the plant C is assimilated by the microbial biomass, which itself is subject to biodegradation and transformation. For many crop residues in temperate climates, 50 to 70% of the original C is lost as CO_2 after 1 year. Of the residual soil C 10 to 20% is microbial biomass and the remainder has become incorporated into new SOM.[24] Approximately 20% of the residual C is associated with the HA fraction in the form of peptides and polysaccharides. The major portion of residual plant lignin C is associated with aromatic complexes.[25] Stott et al.[26] found that the majority of polysaccharide and protein C in wheat (*Triticum aestevum*) residues became associated with the FA fraction of new organic matter. From 36 to 54% of the C derived from wheat straw lignin was found in the HA fraction. Corn (*Zea mays*) residues underwent similar incorporation into new humus fractions.

C. WHOLE SOIL ANALYSIS

A difficulty with the chemical extractions of SOM and fractionation in HA, FA, and humin is that they are tedious and labor intensive and not suitable for large numbers of samples.[27] New approaches that include ^{13}C-nuclear magnetic resonance (NMR) spectroscopy, solid-state cross-polarization with magic angle spinning (CP/MAS-NMR), and pyrolysis-soft field ionization mass spectrometry (Py-FIMS) have been successfully applied to the study of in situ SOM in a number of soils.[27–30] The ^{13}C-NMR spectrum provides specific information on the chemical structures involving ^{13}C atoms within a molecule. The C skeleton of humic materials is observed rather than the adjacent protons, allowing the functional groups to be detected. Carbon nuclei are spread over a wide range of chemical shifts that effectively separate signals even when carbons have only small differences in diverse structural environments.[31] Carbon structures are determined in relative terms from the chemical shifts that occur when energy is absorbed by a molecule spinning in a magnetic field.[31] The types of C which can be detected by ^{13}C-NMR spectroscopy are presented in Table 1. The chemical shift is expressed as parts per million (ppm). The intensity of the signal detected and the spectral quality of that signal (signal:noise ratio) are dependent upon the amount of ^{13}C present in the sample and the concentration of the ^{13}C nucleus present among the wide range of C structures that are found in soil.[31,32] The presence of paramagnetic ions also reduces the efficiency of signal acquisition. The major paramagnetic ion in soil is Fe^{3+} but other transition metal cations may cause signal reduction if present in high concentrations. Baldock et al.[32] reported that the concentration of the ^{13}C nuclei could also be increased by incubating soil with ^{13}C-labeled glucose and following changes in the chemical structure of the substrate carbon followed as it was decomposed by the soil microflora and incorporated into SOM. Oades et al.[33] found that fractionating soils on the basis of particle size and density concentrated organic C and improved the signal quality of solid-state

^{13}C-NMR spectra. Solid state ^{13}C-NMR spectra represents the sum of all organic structures in the sample but cannot differentiate among litter, partly humified, and humified organic matter. It is also not possible, using unfractionated soil samples, to distinguish between structures that are associated to some degree with inorganic components in the soil and those that are not.

Table 1 Types of Carbon which Can Be Detected by ^{13}C-NMR Spectroscopy in Humic Materials and the Corresponding Chemical Shifts

	(ppm)
Aliphatic C (alkanes + fatty acids)	0–40
Protein C, peptide C, amino acid C, C in OCH3	41–60
Carbohydrate C	61–105
Aromatic C	106–150
Phenolic C	151–170
Carboxylic C (total acidity)	171–190
Carbonyl C	210–230
Aliphaticity (%)	$\frac{(0–105) \times 100}{(0–170)}$
Aromaticity (%)	$\frac{(106–170) \times 100}{(0\text{-}170)}$

From Wilson, M.A., *NMR Techniques and Applications in Geochemistry and Soil Chemistry*, Pergamon Press, Oxford, 1987. With permission.

In applications of Py-FIMS, the sample is pyrolyzed under vacuum directly in the ion source of the mass spectrometer, and the volatile components identified by field ionization (FI) mass spectra. The advantages of Py-FIMS and descriptions of the methodology have been summarized by Schulten.[34] Schnitzer and Schulten[35] reported that the dominant fractions obtained from whole soil analysis include carbohydrates, phenols, lignin monomers and dimers, and, to a lesser extent, *n*-fatty acids. Minor components included *n*-alkyl monoesters and diesters, *n*-alkenes, *n*-alkylbenzenes, and N-compounds. It is noteworthy that both Py-FIMS and ^{13}C-NMR provide similar results. In the near future, development of quantitative analyses will greatly improve the application of the information gathered by these techniques to changes in soil C resulting from land management. These methods applied to whole soils avoid structural alterations which occur during extractive methods. They have given useful empirical estimates of the degree of humification as well as suggesting some fundamental aspects of organic matter structure.

IV. PHYSICAL FRACTIONATION OF SOIL ORGANIC MATTER

Although chemical extractions have been routinely used to characterize the chemical structure and composition of SOM, they have not been very useful in identifying specific SOM pools that diminish upon intensive management. The physical occlusion or "protection" of organic materials, restricting the accessibility of microorganisms and enzymes, is believed to be an important mechanism controlling SOM turnover.[18,36,37] Physical fractionation of soil has been more useful in making these distinctions.[38–40] Physical separations address this issue by yielding information on the distribution and concentration of SOM within different parts of the soil matrix. The distribution of organic matter within physical fractions of soil can be measured by disrupting soil structure followed by the separation of fractions by either particle size or density gradients (Table 2).

A. METHODS OF DISRUPTION

Mechanical disruption of soil particles is commonly achieved using either sonication or shaking.[41,42] The principle behind sonic dispersion is the transmission of vibrating sound waves that create microscopic bubbles, which upon collapse produce a high energy of cavitation.[43,44] At this time there are no standard protocols for sonic dispersion. The duration of ultrasonic treatment required to achieve complete dispersion will vary among soil types, soil/water ratio, soil-suspension volume as well as probe dimensions and power output intensity. Christensen[42] stressed that the optimum treatment time should be determined for each soil under study to ensure that dispersion was maximized. The minimum energy at which

Table 2 Comparison of Methods for Physical Disruption and Separation of SOM

Method	Principles	Size Fractions	Advantages	Disadvantages
Disruption				
Sonication	Vibrating sound waves create microscopic bubbles that upon collapse produce a high energy of cavitation that disrupts the bonds of soil aggregates	Particle size distribution	Minimizes chemical transformations	Potential for redistribution of organic matter among size/density fractions; currently no standard protocol
Shaking	Used principally with chemical extraction of whole soil; apparatus include end-over-end, reciprocal, wrist action, and rotary shakers	Particle size distribution	Simple, a wide range of disrupting energies can be obtained	Soil samples having high sand contents increase the rate of disruption compared to heavier textured soils
Chemical	Treatment is commonly used prior to disruption; some chemical dispersants can selectively solubilize or oxidize various OM binding aggregates, e.g., periodate-tetraborate specific for carbohydrates	Particle size distribution	Useful in understanding function of various fractions of organic matter	Some components of organic matter are modified through chemical transformations, solubilization, or oxidation
Separation				
Dry Sieving	Separates soil particles based strictly on size and used primarily in aggregate separations of nondisrupted soil	Particles >50 μm		Sieve abrades aggregates resulting in an average aggregate size below that of the field
Wet Sieving	Separation of aggregates into various sizes through a nest of sieves under water	<2 mm	Useful index of aggregate stability	
Sedimentation	Separates soil particles based on equivalent spherical diameter and used most often with a disruption pretreatment	<1 mm		Major limitation is that aggregates >1 mm in diameter settle too rapidly to be measured accurately
Density	Separates particles based upon the weight per unit volume, independent of size and shape and is used to separate light and heavy fractions; requires centrifugation when working with fine particles	Particle size-fractions with density gradients from 1.6 to 2.2 mg m^{-3}		

cavitation occurs should be used if minimal disruption of soil structure is desired. Low energy levels are difficult to control and require careful calibration. Although seldom done, the sonicator should be calibrated so that results among investigators can be compared. The energy emitted by the probe can be estimated by measuring the downward energy of the probe on a sensitive balance. Christensen[45] showed that a sonication time of 15 minutes and 695 J ml^{-1} (300 W) was generally sufficient for maximal dispersion in three sandy soils (Figure 3). Clay yield increased and silt decreased with increased sonication time, indicating the progressive disruption of silt-sized particles. One of the greatest problems with the use of sonication in SOM studies is the potential for redistribution of organic matter among size/density fractions. Increasing the intensity of sonication results in the recovery of increasing amounts of organic matter in the fine silt and clay fractions. However, low levels of sonication provides incomplete dispersion of soils, causing microaggregates of smaller size particles to be included in silt and sand size separates.

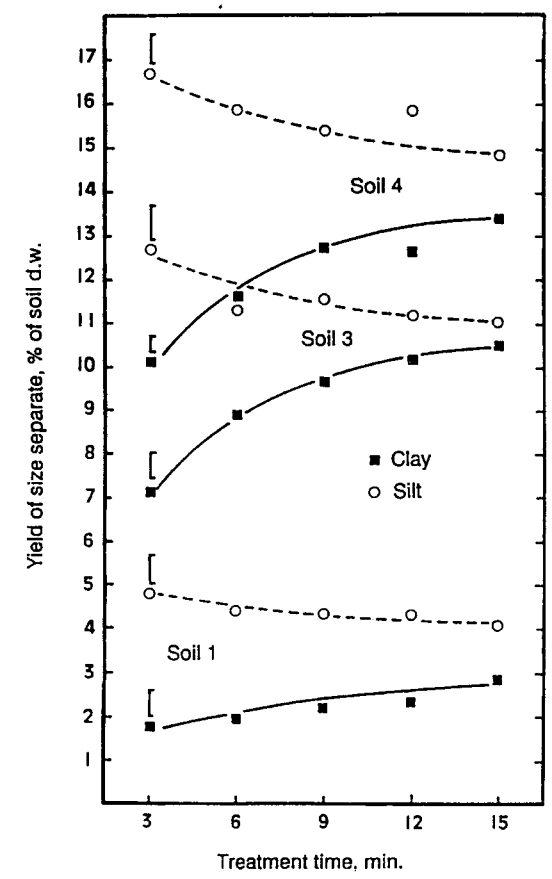

Figure 3 Influence of ultrasonic treatment on yields of clay (<2 μm) and silt (2 to 20 μm) from three Danish soils. (From Christensen, B.T., *Adv. Soil Sci.*, 20, 2, 1992. With permission.)

Shaking is a less intense alternative dispersion method to sonication. Typical apparatus used are end-over-end, reciprocal, wrist action, and rotary shakers.[42] The duration of shaking and addition of chemical dispersants contribute to the uniformity with which soil is disrupted. Introducing glass beads or agate balls enhances the dispersion effect during shaking. The use of chemical dispersants during physical disruption may create changes in some SOM components through chemical transformations, solubilization, or oxidation.

B. METHODS OF SEPARATION

The physical fractionation of soil is generally accomplished using dry or wet sieving, sedimentation, and/or density separations. Dry sieving separates soil particles based upon size and has been used primarily in separating large aggregates of relatively undisturbed soil samples. Dry sieving is often used

for wind erosion studies to determine particle sizes susceptible to wind erosion.[46] The duration of sieving, abrasion, and impact forces influences the final size distribution of aggregates. An indication of how the sieving procedure has affected the aggregate size distribution can be obtained by running the soil through the sieve a second time. Comparison of the amounts of soil in the various aggregate sizes after the first and second sieving provides an estimate of how much the abrasion has changed the aggregate distribution. Wet sieving is used more often than dry to determine particle size distribution and stability of soil aggregates. For primary particle size separations, soil is generally dispersed using sodium hexametaphosphate and NaOH to raise the pH and complex bridging ions to further enhance dispersion of fine particles. Sand fractions are separated on sieves, with silts and clays separated by sedimentation.

Fractionation using sedimentation separates particles that can vary in size, shape, or density based upon their equivalent spherical diameter. Sedimentation dynamics are described by Stokes Law which assumes (1) that terminal velocity of each particle is reached as soon as settling begins, (2) settling and resistance are due to the viscosity of the liquid, (3) particles have similar density and are spherical and smooth, and (4) there is no interaction between settling particles.[43] Density fractionation has historically relied on organic liquids, but aqueous solutions of inorganic salts have become increasingly popular. The use of inorganic liquids is essential where the organic matter content of particle size fractions needs to be determined. The most frequently used organic liquids are tetrabromoethane (2.96 g cm^{-3}), bromoform (2.88 g cm^{-3}), and tetrachloromethane (1.59 g cm^{-3}). Inorganic liquids include polytungstate, sodium iodide (NaI), and sodium bromide (Na_2Br).

Edwards and Bremner[47] proposed that a large portion of primary soil particles (sand, silt, and clay) and organic matter are contained in microaggregates consisting of clay and humified matter linked by polyvalent metals. Tisdall and Oades[38] and Oades[48] present a conceptual model of the association of SOM and soil structure based upon a description of three physical fractions that exist in mineral soils: free primary particles, microaggregates, and macroaggregates. Aggregates are bound together by three types of cementing agents: (1) transient, comprising microbial and plant derived polysaccharides; (2) temporary, which include roots and fungal hyphae; and (3) persistent, aromatic humic material associated with Fe and Al, and other polyvalent metal cations. The metals act as clay-organic matter and organic matter-organic matter bridges. Persistent organic matter binds primary minerals into microaggregates (0.002 to 0.020 mm) which are stable to sonication. These, in turn, are bound by additional persistent organic matter to form microaggregates 0.02 to 0.25 mm in size which disintegrate with sonication but remain intact over long periods of cultivation. Tisdall and Oades[38] further suggested that microaggregates are bound together into macroaggregates (0.25 to 2.0 mm) by transient and temporary organic matter.

Elliott[39] and Elliott and Coleman[49] modified the micro-/macroaggregate model by suggesting that the matter held within macroaggregates has an intermediate turnover time and it is this pool which makes up the bulk of the organic matter lost due to cultivation. As physical disruption energy increases, particle distributions shift to contain smaller particles.[39,50,51] The organic matter associated with the sand fraction released during sonication and sedimentation is composed mostly of the light fraction, i.e., undecomposed organic matter of plant origin.[36,52–54]

Organic matter associated with different primary particles has been shown to have varying rates of turnover.[36,52,55] There is a large body of literature supporting both the quantitative importance and the resistant nature of the SOM associated with the silt/coarse clay fraction and sometimes faster turnover rate of the fine clay separates obtained from sonication and sedimentation.[52,56–60] This, however, may be somewhat an artifact of the fractionation technique in which labile microbial constituents and other compounds become adsorbed upon the fine clays during fractionation. In several studies, Tiessen et al.[61,62] found that the greatest loss of C following 60 years of cultivation was associated with the sand fraction (light fraction). Some of the loss may have been the consequence of the disintegration of aggregates resulting in an accumulation of SOM in finer size fractions. The proportion of SOM in smaller particle-size fractions increased with years of cultivation.

Buyanovsky et al.[63] tracked the distribution of ^{14}C originating from labeled soybean [*Glycine max* (L.) merr.] residues, among vegetative fragments (2 to 0.2 mm and 0.2 to 0.053), the fine silt (25 to 2 μm), clay (<2 μm) fractions, and within natural aggregates (2 to 1, 1 to 0.5, 0.5 to 0.25, and <0.25 mm) over a 4-year period on Sanborn Field, Columbia, MO. Following a combination of wet sieving and ultrasonication, Buyanovsky et al.[63] compared residence times of each fraction to values of the C pools presented in C cycling models of Jenkinson and Rayner[19] and Parton et al.[64] (Table 3). Theoretical pools I and II are characterized by the quality of plant material, and were suggested to be associated with the vegetative fractions >0.053 mm, with turnover times ranging between 0.5 to 2 years. Pool III

represents the soil microbial biomass with a turnover time ranging from 1 to 10 years, which encompassed aggregate sizes ranging from nonaggregated to 2 mm with a turnover spanning 1 to 7 years. Turnover times for pools IV and V were obtained from estimates derived by Balesdent et al.[59] and Hsieh.[81,82] These results provide a link between the physical soil fractions and accepted conceptual models describing soil C dynamics.

Table 3 Comparison of Mean Residence Times (Years) of C in Theoretical Pools of SOM and in Soil Physical Fractions

Pool	Jenkinson and Rayner[19]	Parton et al.[64]	Physical Fractions; Buyanovsky et al.[63]
I	Decomposable plant material, 0.24	Metabolic plant residues, 0.5	Vegetative fragments (2–0.2 mm), 0.5–1
II	Resistant plant material, 3.33	Structural plant residues, 3.0	Vegetative fragments (>0.053), 1–2 Vegetative fragments (0.053–0.025 mm), 2–3 Macroaggregates (2–1 mm), 1–4
III	Soil biomass, 2.44	Active soil C, 1.5–10	Aggregates (1–0.5 mm), 2–10 Aggregates (0.5–0.1 mm), 3–10 Nonaggregated soil, 7
IV	Physically stabilized, 72	Slow soil C, 25–50	Fine silt (internal), 40
V	Chemically stabilized, 2857	Passive soil C, 1000–1500	Fine clay (internal), 1000

From Buyanovsky, G.A., Aslam, M., and Wagner, G.H., *Soil Sci. Soc. Am. J.*, 58, 1167, 1994. With permission.

Christensen and Sorensen[65] reported that after 5 to 6 years of cultivation the highest concentration of C was associated with the clay fraction (66 to 84%), whereas silt accounted for 4 to 19%, and sand less than 2%. The decrease in labile but physically protected organic matter, resulting from aggregate breakdown, reduces the ability of the SOM to supply plant nutrients via mineralization.[66] Fine clay mineral colloids are important in protecting labile SOM, thereby slowing its transformation to recalcitrant forms or loss as CO_2 through decomposition.[18,56] Physical protection of organic matter results from surface adsorption on a molecular scale or by occlusion within microaggregates.[37,67]

Elliott[40] compared aggregate size distributions of native and cultivated soils and found that, as a result of cultivation, the organic matter which was readily lost was that which normally bound organic matter-poor microaggregates into organic matter-rich macroaggregates. He proposed that, if macroaggregates are composed of microaggregates that contain more humified organic matter, plus interaggregate material that has less humic character, then there should be differences in organic matter chemistry between micro- and macroaggregates and that an important pool of organic matter is the glue that cements microaggregates into macroaggregates. Higher concentrations of organic C and N in macro- compared to microaggregates have been reported.[50,51,68,69] Dormaar[51] found greater concentrations of recalcitrant organic matter (resin extractable C), higher HA:FA ratios, and less polysaccharide, polyuronide, and phenol in microaggregates than macroaggregates in cultivated soil.

C. DENSITY FRACTIONS OF ORGANIC MATTER

Based on floatation in high specific gravity liquids, SOM can be divided into two broad categories: (1) a light fraction (LF) consisting of relatively mineral-free, incompletely decomposed plant and animal debris, and associated microorganisms, and (2) a heavy fraction (HF) consisting of organic matter adsorbed to mineral surfaces or contained within organomineral microaggregates.[70]

The LF of soil is usually defined as having a density ranging from 1.5 to 2.0 g cm^{-3}, a relatively wide C:N ratio, and is biologically active with a rapid turnover rate. The HF has a narrow C:N ratio but decomposes more slowly than the LF.[21,43,71] The relatively rapid mineralization rate of LF may be related to the labile nature of its constituents and to the lack of protection by soil colloids. Janzen et al.,[72] in evaluating the LF as a measure of labile SOM, studied three long-term rotations in western Canada. The LF comprised 2 to 17% of the soil organic C and was highest where soils were in perennial forages or continuously cropped and lowest where bare fallow was part of the management. The respiration rate

and microbial biomass N were highly correlated to the LF content. N-mineralization varied somewhat because of some immobilization during decomposition.

Cambardella and Elliott[42] isolated a particulate organic matter (POM) fraction that they suggest corresponds to the characteristics of the intermediate or slow SOM pools described in SOM simulation models.[20,73,74] POM can be isolated by shaking 10 g of soil in 30 ml of 0.5% sodium hexametaphosphate for 15 minutes. The dispersed soil is passed through a 53-μm sieve and rinsed several times with water. The POM fraction retained on the sieve and mineral-associated organic matter passing through the sieve are collected, then dried in a forced air-oven at 50°C overnight, and total organic C from each determined using either dry or wet oxidation techniques. The difference between the C value of the material passing through the screen and that determined from a nondispersed sample is equal to the C retained on the sieve, i.e., POM. Since the sand fraction is removed from the mineral-associated fraction during dispersion, a correction factor is used to obtain a per gram soil percentage.

Cambardella and Elliott[42] compared 20 years of bare fallow, stubble mulch and no-till management with native prairie. Wheat cropping reduced the POM-C fraction from 40 to 18% for plowed wheat, 19% for stubble mulch, and 25% for no-till. Stable C isotope composition of the POM isolated from the no-till and stubble-mulch treatments showed that 87% of the POM-C was derived from the original grassland with only 13% derived from the wheat crop in the plowed treatment. The percent of C derived from wheat increased to 31% under no-till management. Scanning electron microscopy indicated that the POM fraction was composed mostly of root fragments in various stages of decomposition.

D. PROTECTION OF SOIL ORGANIC MATTER WITHIN AGGREGATES

SOM is derived either directly from plant material or from microbial products derived secondarily from the processing of plant material. These materials may be physically protected from microbial access within soil aggregates, be they labile or recalcitrant. In either case the SOM mineralization rate may be reduced. If labile substrates are released from physical protection, mineralization may be rapid, whereas mineralization may be slow where recalcitrant SOM is released from the disruption of aggregates. For these reasons, physical fractionation followed by chemical or biological assays of lability is a useful approach to the study of SOM dynamics. A modified version of the hierarchical aggregate hypothesis of Tisdall and Oades[38] can explain many aspects controlling the rate of SOM turnover in many soils from temperate regions.[75]

POM is a significant component of macroaggregate SOM.[41] Analysis of differences in C and N content and C/N of macroaggregates from different tillage treatments suggests a strong relationship between the stability of the aggregates and the presence of POM.[69] In addition, it was suggested that a greater proportion of wheat-derived POM was found in more intensively cultivated soils. Beare et al.[76] found that POM-N comprised a greater proportion of aggregate N in no-till compared with conventional cultivations and values increased with increasing aggregate size. They suggested that large microaggregates (106 to 250 μm) were formed in conjunction with POM at the center of macroaggregates, as proposed earlier by Oades.[48]

Crushing aggregates releases SOM that is otherwise less available as substrate for microorganisms.[40,77] It is unclear whether this released SOM is of microbial or plant origin but both may be physically protected. Disruption of aggregates from no-till increased mineralization by almost 20% but had much less effect (5 to 10%) in conventionally tilled soils.[78] The effect of crushing on mineralization may be reduced by storage of air-dried soils.

An enriched labile fraction was isolated using a combination of wet sieving to obtain macroaggregates, gentle sonication, particle size separation (2 to 20 μm), and densitometry (2.07 to 2.22 g cm^{-3}).[79] It comprised a significant portion of the total SOM (e.g., 25% of total N in no-till soil) and was considerably more labile than the aggregates from which it was isolated. This observation supports the suggestion that organic matter between microaggregates within macroaggregates is labile but physically protected.[40] Interestingly, soil containing the lowest proportion of this fraction was from native grassland, where a much larger proportion of total SOM was isolated in the POM fraction.

V. TRACER USE IN SOIL ORGANIC MATTER STUDIES

A. CARBON DATING

The bombardment of ^{14}N by cosmic radiation in the atmosphere produces a low level of ^{14}C which when incorporated into plant tissue by photosynthesis provides a long-term tracer for SOM dating (^{14}C half-life is 5568 years). Radiocarbon dating using naturally occurring ^{14}C has become an important tool in

describing SOM dynamics by providing the opportunity to study the distribution of naturally occurring ^{14}C in soils.[80–82] It is particularly useful in characterizing the age of the more resistant fractions of SOM.[83] Above-ground testing of thermonuclear devices in the 1950s and 1960s resulted in the temporary enrichment of atmospheric radiocarbon (Figure 4). These explosions released neutrons that reacted with atmospheric N_2 to produce ^{14}C which also entered the terrestrial carbon cycle.[19,84] Although atmospheric testing was suspended in the 1970s the biosphere maintains a ^{14}C activity about 30% above 1950 values but continues to fall, producing a measurable signal that allows determination of turnover rates in the important 30- to 60-year time interval to be measured[85] (Figure 4).

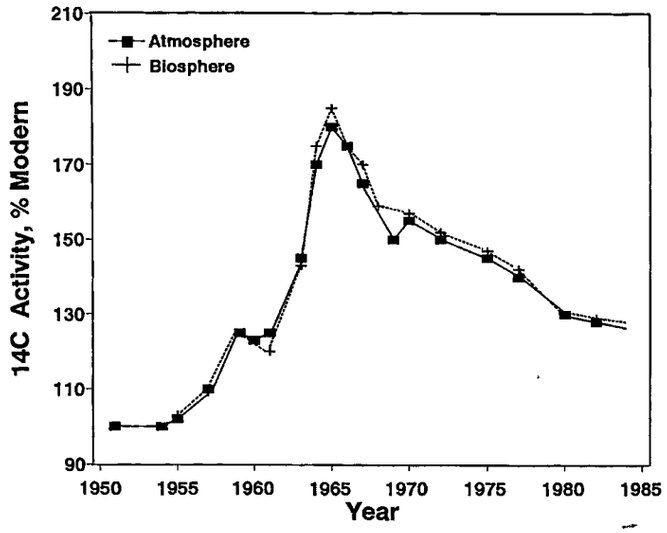

Figure 4 The mean annual specific ^{14}C activity in the atmosphere and biosphere of the Northern Hemisphere, 1950 to 1981. (From Hsieh, Y.-P., *Soil Sci. Soc. Am. J.*, 57, 1020, 1993. With permission.)

Following incorporation of recent plant material into the soil, ^{14}C is diluted by older soil C so that the enriched ^{14}C present in old SOM comprises a smaller fraction of the total C than in the plant material. Soil organic C is a mixture of recently added plant debris and the associated biota and very much older humic materials associated with mineral particles. Therefore, absolute ages of SOM of present-day soils are difficult to determine. The terms radio carbon age or mean residence time (MRT) are most often used. Table 4 provides representative MRTs of total SOM and organic matter fractions for several soils of the Northern Great Plains. Paul et al.[86] found half-lives of 250 to 1900 years for SOM of western Canadian soils. Estimates of the MRT of organic matter in Mollisols of the Central Great Plains range from 2000 to 7000 years.[87]

Martel and Paul[88] measured the ^{14}C content of field soils and compared them to the ^{14}C distribution in a soil incubated with ^{14}C-acetate. Carbon dating showed the MRTs for the surface horizon was 350 years but varied from modern to 1910 years for different chemical fractions. Their work also indicated that SOM in surface horizons turned over more rapidly than lower horizons. Jenkinson and Rayner[19] constructed a model describing the turnover of SOM from soils under long-term management at the Rothamsted Experiment Station in Harpenden, U.K. Using a combination of ^{14}C-labeled plant material and radiocarbon dating of SOM they were able to follow the flux of C through five SOM pools (decomposable plant material, resistant plant material, soil microbial biomass, physically protected, and chemically stabilized organic matter). Ages of these pools ranged from several months for plant materials to several thousand years for chemically and physically stabilized SOM fractions. Campbell et al.[89] estimated compartments for the labile and stable C pools to have turnover rates of 53 and 1429 years, respectively.

Hsieh[81] proposed that the turnover of soil organic C could be described by two major pools, an active pool with a turnover time of less than a few decades and a stable pool exhibiting a considerably longer turnover time. This was based on the assumptions that cultivation causes significant loss in the active but not stable, and that bomb effects apply primarily to active, and not to stable SOM pools. Comparisons between the Morrow plots (Urbana, IL) and the Sanborn Field (Columbia, MO) showed the mean age

Table 4 Representative Mean Residence Times of SOM and Organic Matter Fractions

Site Description	Mean Residence Time[a] (years)	Ref.
Saskatchewan, Canada — Mollisol	1000	86
Saskatchewan, Canada		89
Mollisol	870 ± 50	
Alfisol	250 ± 60	
Saskatchewan, Canada — Catena		
Crest to depression	545 to modern	88
B horizon	700–4000	
Akron, CO, U.S.	2000–7000	87
Soil Fractions		
Unfractionated soil	870 ± 50	89
Fulvic acid	495 ± 60	
Humic acid (total)	1235 ± 60	
Humin (total)	1140 ± 50	
Decalcified soil	1450	19
Acid hydrolysate	515	
Residue from hydrolysis	2560	
Humin	1240	

[a] MRT = 18,500 $\log_{10}$ (A_o/A), where A_o is the activity of the modern standard (NBS oxalic acid) and A is the activity of the unknown sample.

of the stable SOM pool at both sites was much greater than a few hundred years at 2973 and >600 years, respectively. These data confirmed that the stable SOM pool is very resistant to biodegradation. Hsieh[82] also demonstrated that the MRT of the active SOM pool in surface soils could be determined using bomb effect ^{14}C if the yearly variation of ^{14}C level in the atmosphere was known.

B. NATURAL ^{13}C ABUNDANCE

The difference in assimilation of ^{13}C between plants having C_3 vs. C_4 photosynthetic pathways provides an additional approach for assessing the long-term stability of SOM.[90] The stable isotope ^{13}C occurs naturally in the atmosphere at a concentration of 1.1% ($\delta^{13}C = -7‰$).[91] During photosynthesis C_3 plants incorporate less $^{13}CO_2$ than do C_4 plants.[92,93] The ratio of ^{13}C and ^{12}C of plants or SOM are reported as $\delta^{13}C$ values measured relative to a standard:

$$\delta^{13}C‰ = \frac{(^{13}C/^{12}C \text{ sample} - {}^{13}C/^{12}C \text{ standard})}{^{13}C/^{12}C \text{ standard}} \times 10^3 \quad (1)$$

The standard was initially a limestone fossil (*Belemnitella americana*) from the Cretaceous Pee Dee formation of South Carolina.[94] This PDB standard is no longer available; however, other standards calibrated against PDB can be obtained from the National Institute of Standards and Technology (NIST) or the International Atomic Energy Agency (IAEA).

The $\delta^{13}C$ values of C_3 plants range from –23 to –40‰, with many occurring at about –26‰ (Figure 5). The C_4 plants have a $\delta^{13}C$ range from –9 to –19‰ and most often are found to occur at –13‰.[95–97] Plants with the Crassulacean acid metabolism (CAM) pathway often have a wide range between the two major types of vegetation. These however, are restricted to desert vegetation. Since the range of C_3 plants does not overlap the range of C_4 plants, differences in isotope ratios can be used to quantify the contribution of each photosynthetic pathway to SOM in mixed plant communities.[98,99] Since there is little further discrimination during microbial attack and humification, SOM has a C isotopic composition comparable to that of the source plant material prior to humification. With each change in vegetation between C_3 and C_4 plants, there is a corresponding change in the ^{13}C value of SOM.[100–102] This allows fairly short-term (<5 years) changes in SOM accumulation and turnover to be measured. The ^{13}C technique can now be conducted by readily available automated mass spectrometers. This equipment is cheaper and more reliable than the dating techniques necessary for ^{14}C analyses.

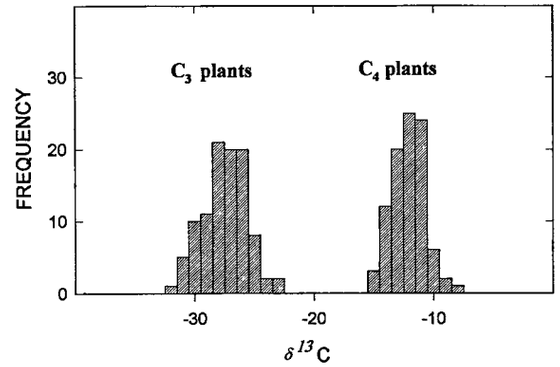

Figure 5 Frequency distribution for a collection of C_3 and C_4 plants depicting the variation in $\delta^{13}C$ composition of photosynthetically fixed carbon. (Adapted from Deines, P., *Handbook of Environmental Isotope Geochemistry*, Vol. 1, Fritz, P. and Fontes, J.C., Eds., Elsevier, Amsterdam, 1980, 329.)

Natural ^{13}C abundance has been used to follow SOM transformations associated with land management.[59,90,103–106] Changes in the natural abundance of different organomineral fractions (micro- and macroaggregates) from soil surface horizons can be used to identify changes in SOM resulting from intensive management. Balesdent et al.[59] measured SOM turnover for a forest soil interplanted to continuous corn for 23 years. They reported that the cropping of a C_4 plant on soils that had been historically dominated by C_3 plants (Hardwood forest) could be used as in situ labeling of SOM. Balesdent et al.[90] demonstrated that the turnover time of the stable SOM pool of the surface soil of the Sanborn Field was >600 years which remained virtually unchanged after decades of cultivation. Since forests yield a C_3 signal, the growth of corn or sorghum (C_4) in any of the Eastern North American sites provides a usable signal. Grasslands vary in their ^{13}C content from the –26‰ of the C_3 grasslands (cool grasslands) to –14‰ from areas such as Kansas where C_4 grasses (warm season) predominate. Most Corn Belt cultivated soils have a label of approximately 20‰. With the accuracy of present-day mass spectrometers it should be possible to follow SOM dynamics from 2 to 4 years of continuous corn (C_4) following C_3 crops such as wheat or soybeans or vice versa in most soils of the central United States.[107]

Carbon dating with ^{14}C, ^{13}C analyses, and extended incubation of soils from historical plots can be combined to determine pool sizes and fluxes required in C balance calculations. Paul et al.[107] found that 70% of the total soil C in 85-year cultivated wheat plots at Akron, CO, was derived from the native prairie vegetation. Carbon dating showed the residue of acid hydrolysis to constitute 56% of total C and to date over 2600 years.

The power of the ^{13}C natural abundance technique is demonstrated using some preliminary data from a never-tilled grassland and corn-soybean plots in southwestern Michigan (Paul, E.A., unpublished). The present day grassland, cleared from a woodlot circa 1950, shows an oak-hickory forest and C_3 grass heritage with a ^{13}C content of –25.5‰ (Table 5). The 80 to 100-year cultivated field with 60% as much total C has a ^{13}C content of –20.9‰. On the basis of a residue return of two parts corn to one part soybeans plus wheat (–17‰), one can calculate that 42% of the total C remaining in the cultivated soil is derived from precultivation SOM. However, 67% of the residue of acid hydrolysis was derived from the precultivation SOM. Carbon dating shows the grassland to reflect bomb ^{14}C inputs at 1.07 ^{14}C pm C. The residue of hydrolysis at 0.98 pm C has an overall MRT of 170 years. The corn-soybean soil shows an overall C dating age of 546 years. The 56% of the C that is nonhydrolyzable dates 1435 years. Reviews of the methods and protocols describing the use of carbon isotopes can be found in Carbon Isotope Techniques edited by Coleman and Fry[108] and Theory and Application of Tracers by Schimel.[109]

VI. FUNCTIONAL DESCRIPTIONS OF SOIL ORGANIC MATTER

SOM is made up of a range of materials that degrade at variable rates due to their chemical composition as well as the degree of protection by absorption to minerals or entrapment within aggregates. Since SOM transformations are controlled by the soil biota, emphasis in this section will be given to analytical approaches that identify changes in active SOM pools or in pools defined by their C-mineralization rates. Microbial biomass has a unique position within the active SOM pool since it is both a source and sink

Table 5 Carbon Dynamics of Soil from a Perennial-Grassland and Corn-Soybean Field on the W.K. Kellogg Biological Station, Long-Term Ecological Research Site (Michigan State University) as Determined by ^{13}C Analyses, C Dating, and Acid Hydrolysis (E.A. Paul, unpublished)

	Grassland		Corn-Soybeans	
	Total	Hydr. Res.[a]	Total	Hydr. Res.[a]
C μg^{-1}	15000	7950 (53%)	9000	5040 (56%)
δ $^{13}C‰$	–25.5	–27.8	–20.9	–24.4
%C from native	—	—	42	67
14C pmC	1.07	0.98	0.93	0.084
MRT[b] (y)	—	170	546	1435

[a] Fraction resistant to 6 *N* HCl acid hydrolysis.
[b] Mean residence time.

of soil C, as well as other important nutrients.[110,111] Microbial biomass and its activity are usually positively correlated with SOM due to a dependence on both the quantity and quality of degradable C sources.[112,113] Biomass C typically comprises 1 to 4% of the SOM and represents a significant part of the active SOM pool.[111,114] The size of this pool and its rate of turnover has been related to shifts in agricultural management,[113,115] differences in soil chemical and physical characteristics,[116] as well as climatic variables.[117]

The energy flux through the microbial biomass drives decomposition of residues and detritus and indicates whether the system is building or depleting the SOM pool.[118] If the amount decomposed exceeds C inputs, SOM will decline and C and N will be lost from the system. The change in the SOM pool is dependent upon the quality and quantity of C inputs, the fluctuation of microbial biomass, and the rate of decomposition.

A. DETERMINATION OF MICROBIAL BIOMASS C

Of the three current most frequently used chemical methods to determine microbial biomass, two are based upon the technique of Jenkinson and Powlson,[110] in which soil is subjected to chloroform fumigation then either (1) incubated for a 10- to 20-day period at which point respired CO_2 is determined or (2) following fumigation, soil is immediately extracted with potassium sulfate.[119] The third method is based upon the addition of saturating quantities of a readily available substrate, such as glucose, that causes a large immediate increase in CO_2 respiration.[120] Table 6 presents a brief comparison of these methods.

1. Chloroform Fumigation-Incubation (CFI)

Beakers of moist sieved soil are placed in a desiccator with a beaker containing 50-ml ethanol-free chloroform. The desiccator is evacuated until the chloroform boils and then placed in the dark for 24 hours, at room temperature. Following the fumigation the beaker of chloroform is removed and the desiccator evacuated to remove chloroform vapors. Fumigated samples and nonfumigated control samples are inoculated with 1 ml of an untreated soil-water suspension, then placed in a 500-ml wide-mouthed jar containing a vial of water and a 5-ml vial of 1 *N* NaOH to trap respired CO_2. The original procedure of Jenkinson and Powlson[110] used 150 g of soil; since then the amount of soil used in the analysis has continued to decline. Our laboratory routinely uses a 25-g soil sample and a 7-day preincubation. The preincubation period reduces the CO_2 flush resulting from sample handling and sieving.

During incubation microbial cells lysed by chloroform fumigation are mineralized at a fairly constant rate that represents about two fifths of the biomass-C in the sample. Biomass is calculated from the expression

$$C_M = (CO_{2(F)} - CO_{2(NF)})/k_C \quad (2)$$

where k_C is the proportion of microbial-C mineralized to CO_2, usually taken as 0.41,[120] $CO_{2(F)}$ is the quantity of CO_2 evolved from the fumigated sample in the 10 days following incubation, and $CO_{2(NF)}$ the amount produced from the nonfumigated sample for the same period. Disturbance caused by sieving of the soil prior to incubation often stimulates microbial respiration resulting in CO_2 values of the control

Table 6 Comparison of Methods Used for Determination of Microbial Biomass in Soil

Method	Principles	C Determination	Advantages	Disadvantages
CFI[a]	20–50 g of soil is fumigated in ethanol-free chloroform atmosphere for 1–5 d, incubated for 10 d; nonfumigated controls are incubated for 10–20 d Liberated CO_2 is measured directly in the headspace or trapped in NaOH	Headspace CO_2 determined by gas chromatography or infrared gas analyzer Trapped CO_2 determined by titration, $BaCl_2$, or dual endpoint acid-base	Reference method Simple and equipment readily available	Use of appropriate control, interference from nonmicrobial labile C
CFE	Similar to CFIM, except following fumigation samples are extracted with 0.5 M K_2SO_4 (5:1 extractant to soil) Total soluble C determined in both fumigated and non-fumigated soil extracts	C determined on suitable automated C-analyzer Wet-combustion, either dichromate or persulfate digestion, CO_2 determined by titration	Low interference from non-microbial labile C Shorter analysis time	Poor correlation to CFIM Chemical disposal
SIR	Glucose amendments are added to soil to determine the lowest concentration that will give the maximum respiratory response Hourly CO_2 evolution is measured to encompass increases, decreases or lags in CO_2 flux	Headspace CO_2 determined by gas chromatography or infrared gas analyzer	Simple, short analysis period (1–3 h) Needs to be calibrated to other methods	

[a] CFI — chloroform fumigation-incubation method;[110] CFE — chloroform fumigation-extraction method,[119] SIR — substrate induced respiration.[120]

greater than that of the fumigated soil. Therefore, the CO_2 flush may need to be calculated using CO_2 respired between 10 and 20 days of the unfumigated soil. Calculations without using a control may give values that are too high but these are highly correlated to biomass estimates determined by direct microscopy.

2. Chloroform Fumigation-Extraction (CFE)

The CFE method is based upon the fumigation technique but microbial constituents released by fumigation are extracted directly. Soil samples are prepared as described above in containers suitable for fumigation and extraction. The length of fumigation ranges from 1 to 5 days dependent upon soil texture. Fumigation efficiency varies with clay content.[121] Therefore, Horwath and Paul[122] recommend up to 5-day fumigation for soils with a high clay content. Extraction of the fumigated and nonfumigated control soil is done with 0.5 *M* K_2SO_4. After shaking the suspension is filtered and the filtrate stored frozen until analyzed. Extracted C can be determined either by wet combustion using potassium dichromate or persulfate digestion procedure or on automated soluble C analyzers.[123] Liberated CO_2 is trapped in 1 *M* NaOH and titrated with HCl.[3] Total N rendered extractable by fumigation can also be determined using the autoclave/persulfate digestion method of Cabrera and Beare[124] on the same extracts used for extractable-C determinations. Biomass C is calculated from the equation

$$C_M = (C_F - C_{NF})/k_{ec} \quad (3)$$

where, C_F and C_{NF} are μg C determined in fumigated and nonfumigated samples, respectively, and k_{ec} is the proportion of microbial-C extracted from soil, usually taken as 0.35.[121]

3. Substrate-Induced Respiration (SIR)

The SIR method is based upon the addition of saturating quantities of a readily available substrate, such as glucose, that causes a large immediate increase in CO_2 respiration.[120] The CO_2 flush prior to new synthesis of biomass is used as an index of soil microbial biomass. The first step is to determine the lowest glucose level that will give the maximal CO_2 production. The range of glucose amendments is typically 25 to 400 μM g^{-1} soil. Soils high in organic matter will require greater concentrations of glucose addition. In the original procedure glucose was added in a dry form; however, West and Sparling[125] suggested using glucose in solution to enhance substrate dispersion and reduce water limitations during incubation. Samples are amended in gas tight bottles and incubated at 22°C for up to 3 hours. Respired CO_2 is determined using a gas chromatography or an infrared gas analyzer. Results are commonly expressed as CO_2 h^{-1} g^{-1} soil dry weight. Biomass C is determined from the expression of Anderson and Domsch;[120]

$$C_m = 40.04y + 0.37 \quad (4)$$

where, C_m is the microbial biomass and y is the maximal rate of CO_2 respiration. This equation is valid for SIR incubations done at 22°C. To obtain further descriptions of these and other methods used in determining soil microbial biomass, readers are directed to the reviews of Smith and Paul[118] and Horwath and Paul.[122]

B. C-MINERALIZATION POTENTIALS AND POOL SIZE DETERMINATIONS

Mineralization of SOM plays a fundamental role in soil fertility through the release of nutrients and subsequent influence on net primary productivity. The measurement of CO_2 evolution from soil has been widely used to determine the effect of environmental variables on the oxidation of SOM. The C-mineralization coefficient, i.e., the percentage of total organic C evolved as CO_2, has been used to compare soils under varying management.[115,126,127]

Mineralizable soil C can be measured by incubating soil samples in gas-tight containers. At periodic intervals headspace CO_2 can be determined using a variety of techniques, e.g., infrared gas analyzer, gas chromatograph, or NaOH traps.[3] In the latter case trapped CO_2 is precipitated by the addition of $BaCl_2$ and back titrated with HCl. This technique also provides samples for ^{14}C studies. The analysis of CO_2-C has also been automated using continuous flow gas chromatography.[128,129] Zibilske[3] provides an excellent review of the various methods and apparatus used in both field and laboratory settings.

Long-term incubations (>200 days) of soil with measurements of the CO_2 evolved have been widely used to differentiate functional C pools in soil. This method constitutes a biological fractionation of organic matter, whereby the most labile fractions are the most rapidly depleted by the soil microorganisms and subsequent soil C are more slowly mineralized. By analyzing the CO_2 release rates, a variety of mathematical models can be fit to derive estimates for functional C pool sizes and their turnover rates.

The most commonly used models are based on the assumption of first-order kinetics, i.e., where the rate of C mineralization is directly proportional to the amount of C in the organic matter pool. When integrated over time this produces an exponential decay curve. However, in most cases the cumulative CO_2 release curve is analyzed. Models (Table 7) that have been used to analyze incubation data often include two or more first-order components.[107,130–132] Alternatively, "mixed order" models, which include a zero-order (i.e., a constant rate of C mineralization independent of pool size) term for a recalcitrant soil fraction and first-order terms for more labile pools, have been proposed.[131–133]

Most incubations of unamended fresh soil show an initial rapid release of CO_2 as labile organic materials are mineralized. The degree of disturbance involved in sample preparation (e.g., sieving, wetting of dried soils) can influence the initial mineralization flush and therefore differences in sample preparation need to be considered in comparing studies.[134,135] Microbial biomass, which typically declines over the course of the incubation, appears to contribute a substantial portion (20 to 40%) of the C (and N) released.[133,136–138] The initial mineralization flush is often well described by the first-order model while subsequent C release generally follows a linear or slower exponential pattern.

An example of the use of incubation data to compare C pool sizes and turnover rates in soils with different management histories is given in Table 8. Soil from never-tilled grassland, a corn-soybean field, and a 4-year-old reversion plot (abandoned from cropping) were incubated for 200 days at 25°C. The CO_2 released over the 200-day incubation was described as the sum of three first-order rate reactions. The size of the old resistant pool was shown by acid hydrolysis to comprise 53% of the total C in the grassland and 56% in the corn-soybean rotation. Since the residue of acid hydrolysis dated greater than

Table 7 Commonly Used Statistical Models to Estimate Pool Sizes and Rate Constants from Cumulative CO_2 Data

Designation	Integral Form	Parameters (and Units)
Single first order	$C_m = C_0(1-e^{-kt})$	C_0 — mineralizable C pool (mass or concentration) k — specific rate of mineralization ($time^{-1}$)
Double first order	$C_m = C_1(1-e^{-k_1t}) + (1-e^{-k_2t})$	C_1 — labile C pool (mass or concentration) C_2 — resistant C pool (mass or concentration) k_1 — specific rate of labile C mineralization ($time^{-1}$) k_2 — specific rate of resistant C mineralization ($time^{-1}$)
Mixed order	$C_m = C_1(1-e^{-k_1t}) + at$	C_1 — labile C pool (mass or concentration) k_1 — specific rate of labile C mineralization ($time^{-1}$) a — mineralization rate from nonlabile C (mass $time^{-1}$)

Note: In all models, C_m is cumulative CO_2-C and t is time.

150 years, it was assumed that negligible amounts of the CO_2 evolved in 200 days was derived from the resistant pool. This assumption made it possible to analyze the CO_2 data as the sum of two first-order rate reactions. The C mineralized represented 7.3% of total C in the grassland soil which had approximately 50% more organic C and the highest amount of C mineralized. More significant is the mineralization response of the reversion plot which showed a much greater labile C pool compared to the corn-soybean field, despite having the same total soil C. The greater C_1 pool and the higher rate of mineralization of the intermediate (C_2) pool suggest that organic matter is accumulating in the reversion plots. This accumulation was detected in the more labile fractions, but not in the total C values. The 200-day incubation and kinetic analyses indicate that the MRT of the C_2 pool for the three cases were different, ranging from 5 to 10 years.

Table 8 Kinetic Analysis of CO_2 Mineralization Curves for Three Treatments of the W.K. Kellogg Biological Station, Long-Term Ecological Research Site (Michigan State University)

	Grassland	Reversion	Corn/Soybeans
Total C (C_T) ($\mu g\ g^{-1}$)	15,000	9500	9500
C mineralized (C_m) ($\mu g\ C\ g^{-1}\ 200\ d^{-1}$)	1100	930	560
C_m/C_T (%)	7.3	9.8	5.9
Microbial C ($\mu g\ g^{-1}$)	345 (51)	251 (38)	141 (22)
Microbial C/C_T (%)	2.3	2.6	1.5
Pool size (C_1) ($\mu g\ g^{-1}$)	623 (103)	404 (42)	170 (24)
Mineralization kinetics			
C_1^a/C_T (%)	4.1	4.2	1.8
k_1 d^{-1}	0.022	0.029	0.024
k_2 d^{-1}	0.00035	0.00052	0.00029
k_1 MRT (d)	45	34	41
k_2 MRT (y)	7.8	5.3	9.5
R^2	0.997	0.999	0.999

[a] C_1 corrected for microbial growth.

Note: Standard error of the mean shown in parenthesis.

From Paul, E.A., Horwath, W.R., Harris, D. et al., in *Soils and Global Change*, Lal, R., Kimble, T., Levine, E., and Stewart, B.A., Eds., Lewis Publishers, Boca Raton, FL, 1995, 297.

Although laboratory incubations provide for controlled conditions with uniform samples, they present problems with interpretation and extrapolations to in situ mineralization rates. The length of incubation used can substantially affect the parameter estimates for a given model and there is a high inverse autocorrelation of the rate constant (k) and pool size (C_o) using the first-order model.[135] Thus, pool size estimates are highly sensitive to relatively small deviations in the rate constant. To avoid these difficulties in model interpretation, Campbell et al.[139] proposed combining the two parameters (i.e., $k*C_o$) into a single index of mineralization potential. The calculation involved comparing treatment effects on N mineralization. In addition, manipulation of soil (i.e., sieving) changes characteristics of gas exchange,

soil water, and makes available to microorganisms a portion of the organic matter which is less available under field conditions. Consequently, laboratory incubations overestimate C mineralization rates in the field. Despite these limitations, incubation procedures provide one of the most widely used and functionally meaningful measurements of the bioavailability of SOM.

VII. SUMMARY

A wide variety of techniques have been applied to the measurement of soil organic C (SOC). These generally have not been standardized, creating difficulties in comparing and interpreting SOC data across diverse soil types or management practices. In this review we have described a suite of methods that encompass both classical approaches of extraction and identification of specific chemical compounds to approaches that provide functional descriptions of SOC pools and their dynamics. Physical separations yield information on the distribution and concentration of SOM within different fractions of the soil matrix, which combined with functional approaches provide reliable information for estimating both labile and passive SOC pool sizes and turnover. The wealth of information generated by the combination of approaches will improve our understanding of how land management influences the quantity and quality of various SOM pools important in the cycling and release of plant nutrients or the quantification of ecosystem carbon budgets.

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Chapter 4

Crop Residue Input to Soil Organic Matter on Sanborn Field

G.A. Buyanovsky and G.H. Wagner

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I. INTRODUCTION

The value of soil organic matter (SOM) has been long recognized. From earliest times, its level in soil was used as a general indicator of soil productivity. Maintaining or increasing SOM was the goal of many early research projects. When J.W. Sanborn established the plots that bear his name, interest in rotations and the practice of manuring were part of a management philosophy which sought to find methods for increasing SOM and the nitrogen (N) contained therein in order to increase yields.[1] Soils research at Missouri in the early years of this century, however, did not achieve practical success in this matter. After some 35 years of accumulating data on Sanborn and other experimental fields, nearly all plots, including heavily manured ones, showed N losses. With a continued view toward improving management that recognizes the value of SOM, there was a philosophical shift at that time to a consideration of the turnover of SOM and toward N turnover in relation to productivity of the soil.[2] Over the years, as sound soil management has developed, we have continued to keep an appropriate focus on both the level and the dynamics of SOM.[3]

A major factor contributing to the level of SOM is annual input of plant residues. The productivity of native vegetation, tallgrass prairie in this region, once defined the annual carbon (C) input. Characteristic organic matter levels in soils were controlled by these vegetative inputs and by other interrelated factors such as climate and the nutrient release from the soil parent material. Under cultivated agriculture, crop residues serve as C inputs and thus influence both the level and the dynamics of organic matter in soil.

The level of SOM can be altered by changes in the ecosystem. The original native equilibrium levels were established as the soils developed over hundreds and thousands of years. In most cases, the current

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