Video Article

Assessment of Labile Organic Carbon in Soil Using Sequential Fumigation Incubation Procedures

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Abstract

Management practices and environmental changes can alter soil nutrient and carbon cycling. Soil labile organic carbon, a readily decomposable C pool, is highly sensitive to disturbance. It is also the primary substrate for soil microorganisms, which is fundamental to nutrient cycling. Due to these attributes, labile organic carbon (LOC) has been identified as an indicator parameter for soil health. Quantifying the turnover rate of LOC also aids in understanding changes in soil nutrient cycling processes. A sequential fumigation incubation method has been developed to estimate soil LOC and potential C turnover rate. The method requires fumigating soil samples and quantifying CO_2-C respired during a 10 day incubation period over a series of fumigation-incubation cycles. Labile organic C and potential C turnover rate are then extrapolated from accumulated CO_2 with a negative exponential model. Procedures for conducting this method are described.

Video Link

The video component of this article can be found at https://www.jove.com/video/54614/

Introduction

Due to its vital roles in carbon (C) and nutrient cycling and its sensitivity to soil change, soil LOC is an important parameter to measure as an indicator of soil organic matter quality. Forests and agroecosystems to a large degree depend on the mineralization of nutrients in soil organic matter as a source of nutrients. Management activities can change the pool size and turnover rate of soil organic C, resulting in changes in nutrient supply[1]. Soil organic C consists of two primary fractions of recalcitrant C, which has turnover rates of several thousand years, and LOC, which has turnover rates from a few weeks to a few years[2,3,4]. Soil labile C consists of readily decomposable substrates such as microbial biomass C, low-molecular-weight compounds (amino acids, simple carbohydrates) from plant rhizodeposition, and decomposition byproducts and leachates from plant litter[1,4,5]. Because soil labile C is readily decomposable, it is highly sensitive to management practices and natural phenomena that disturb or alter soil[6]. Soil labile C serves as the primary energy source for soil microorganisms in the decomposition of organic matter[7]. As such, LOC impacts nutrient cycling to a greater degree than does stable forms of soil organic C[8]. Soil microorganisms are also responsible for the majority of heterotrophic respiration that occurs during decomposition of recalcitrant soil organic matter facilitated by the priming effect of LOC[9,10,11]. This respiration plays a substantial role in global C cycles because soil organic C is approximately double that of atmospheric C[11].

As a result of its importance in terrestrial ecosystems, several methods have been developed to estimate soil LOC. These methods can be delineated into three general classifications: physical, chemical, and biochemical. Densitometric separation methods are physical methods that consist of separating soil organic C into heavy or light fractions or into coarse and fine particulate organic C[12,13,14,15]. Separation methods are relatively easy to perform, but they do not often produce consistent results because these fractions vary with soil type mineral composition, plant material size and density, and soil aggregate consistency[13,15]. Separation methods also produce only quantitative information about LOC[15].

Several chemical methods are available for LOC estimation. Aqueous extraction of organic carbon is relatively easy to perform, and the methods often provide easily reproducible results. However, these extractions do not involve the whole spectrum of available substrates for microorganisms[15]. Several oxidation methods for chemical fractionation of soil organic C have been developed. Oxidation methods have the advantage of characterizing the quantity and quality of labile organic C, although some methods require work with hazardous chemicals and there is variability among the methods in reproducibility of results[15]. The acid hydrolysis extraction method is another type of chemical fractionation procedure that can measure the quantity and quality of LOC, but results of this method do not facilitate interpretation of its biological properties[13,15].

Biochemical methods for interpretation of soil LOC have been developed. Labile organic C can be measured as CO_2 released by microorganisms in respiration assays. These assays provide estimates of true mineralizable organic matter, but typically only the most labile compounds are mineralized during the assays[15]. Soil microbial biomass C measured by fumigation-incubation[16] and fumigation-extraction[17] has

been used to develop inferences about LOC. However, these procedures provide estimates of C in microbial biomass rather than LOC. Both fumigation procedures include subtraction of values from non-fumigated soil to determine microbial biomass C, but it has been suggested that values obtained without subtraction of non-fumigated soil provide a measure of labile organic fractions of C in addition to microbial biomass[18].

The sequential fumigation-incubation (SFI) procedure[13] for measuring LOC is a biochemical method adapted from the fumigation-incubation procedure[16] for soil microbial biomass C measurement. The SFI method has some advantages relative to other methods of estimating LOC. A conceptual basis for the method is that LOC is the microbially degradable C that governs microbial growth and that LOC is physically accessible and chemically degradable by soil microorganisms. Under field conditions, microbial growth is typically limited by carbon availability, nutrient availability, available pore space, and/or predation. These factors are nearly eliminated by fumigation, creating unimpeded conditions for microbial growth. No nutrients are removed during the incubation period of the method. Over the course of multiple fumigation and incubation cycles, microbial growth becomes limited by C quantity and quality (lability)[13]. The accumulated CO_2 respired during the incubation cycles is used to extrapolate LOC with a simple negative exponential model[11,13,19]. The potential C turnover rate can also be derived from the slope of the exponential model, so the SFI method has the advantage over most other LOC methods of simultaneously estimating the concentrations and potential turnover rate of LOC[11]. For other methods, information on the potential turnover rates of LOC can only be ascertained if tracers such as ^{14}C are used[13]. The SFI method is thus a relatively simple and inexpensive technique for obtaining measurements of both LOC and its potential turnover rates.

Protocol

1. Collect Soil to Get Samples Representative of Conditions within the Experimental Area and within Experimental Units[20]

1. Identify any differences in site properties such as slope and soil properties including texture, bulk density, pH, organic horizon depth, and/or nutrient concentrations. Identify any differences in vegetation type within plots. Use known or published estimates of coefficients of variation for site properties to estimate the number of samples required to attain a pre-specified relative error.
2. Sample soil using an auger or other collection device in a pattern based on site and experimental unit conditions.
    1. For homogeneous conditions, use a random sampling pattern within each experimental unit.
        1. Assign sample points at either completely random locations within the experimental unit or in a zigzag pattern.
        2. Sample soil at each random point or at points assigned in a zigzag pattern. Brush aside organic matter from the surface of mineral soil before using the auger or other collection device to excavate soil sample.
        NOTE: The SFI method was developed using soil horizons from the Oa and below[13]. Further testing is necessary if horizons above the Oa can be tested using the SFI method.
        3. Combine all samples collected within the experimental unit into a single container and physically mix the individual samples within the container to create a composite sample for each experimental unit.

    2. For heterogeneous conditions, which are much more common, use a systematic sampling pattern within each experimental unit.
        1. Sample soils along the transect in the center of each experimental unit such that the distance between sample points within the transect is smaller than the distance needed to represent variability within the experimental units.
        2. Sample soils along multiple transects within each experimental unit that form a grid pattern in relatively large experimental units or experimental units with multiple sources of variability.
        3. Combine all samples collected along each transect into a single container and physically mix the individual samples within the container to create a composite sample for each transect.

2. Prepare Soil for the SFI Assay

1. Place samples in an ice-pack filled cooler immediately after collection in the field.
2. Upon arrival at the facility at which samples are to be stored until analysis, place samples in a refrigerator at 4 °C until sample preparation and SFI procedures are conducted.
3. Sieve soil samples through a 6.4 mm x 6.4 mm mesh sieve. Clean the mesh with water between each sample to prevent contamination between samples.
4. For each sample, measure three 100 g subsamples and place the 100 g subsamples in a 250 ml beaker. Cover each beaker with Parafilm and leave them on a countertop for 10 days at 25 °C.

3. Take Subsamples for Oven-dry Weight Determination

1. At the end of the 10 day pre-incubation of soil samples, remove Parafilm from each sample.
2. Record the weight of an aluminum weigh boat. Take 1 g of soil from all samples and place in weigh boat.
3. Record the weight of the moist soil and weigh boat.
4. Place the weigh boats with soil in an oven at 105 °C. After the samples reach a constant weight, which is typically after 48 hr, record weights of the weigh boats and soil.
5. Subtract weigh boat weight from the weights taken of the moist soil and dry soil within the weigh boat to get moist and dry soil weight. Derive the dry:moist soil ratio by dividing dry soil weight by the moist soil weight.

4. Fumigate Soil Samples

1. Place a damp paper towel in the bottom of at least two (more may be necessary depending on the number of samples) 10.5 L glass vacuum desiccators with porcelain plates.
2. For all samples, weigh 30 g of soil into three separate glass vials. Use vials large enough to hold 40 g of soil and narrow enough to fit within a 40 mm opening if the incubation container design described in section 5 is used.
3. If using labeling tape to identify each 30 g soil subsample, use pencil because fumigation degrades ink.
4. Place two of the three 30 g subsamples for each soil sample into a vacuum desiccator for fumigation and one subsample into a vacuum desiccator that will not conduct fumigation.
5. In a 100 ml beaker, place a layer of boiling stones sufficient to cover the bottom of the beaker.
6. Pour 50 ml of ethanol-free chloroform ($CHCl_3$) into the 100 ml beaker with a layer of boiling stones. Place the 100 ml beaker with boiling stones and $CHCl_3$ in the center of a desiccator filled with the 30 g soil subsamples. Conduct this step under a fume hood.
7. Under a fume hood, use a vacuum to boil the $CHCl_3$ to fumigate two sets of subsamples per soil sample.
    1. Connect the vacuum to the vacuum desiccator with vacuum tubing. Start the vacuum and watch as $CHCl_3$ begins to boil.
    2. Allow $CHCl_3$ to boil for 30 sec and disconnect the vacuum tubing from the desiccator to allow air to flow back into the desiccator. This step promotes $CHCl_3$ gas entry into the soil samples. Repeat twice.
    3. Perform a fourth and final boil of $CHCl_3$, allowing it to boil for 2 min.
    4. With vacuum still running, close the seal on the vacuum desiccator so that the vacuum within the desiccator is maintained. Turn off the vacuum and disconnect the vacuum tubing from the desiccator.

8. Seal the desiccator containing the non-fumigated samples by placing a lid on the desiccator and sealing the vacuum stopper. Place the desiccators (fumigated and non-fumigated) in a darkened area (such as a cabinet) for 24 hr. Do not repeat the vacuum procedures of subsection 4.7 on the desiccator containing non-fumigated samples.

5. Assemble Containers for Soil Sample Incubation

1. Push a 15 cm length glass rod through a size 10 rubber stopper with a hole drilled in the center. The rod diameter should be sufficient to fit through the hole snugly.
2. Label 0.5 L translucent wide mouth polypropylene bottles with identification that corresponds to the fumigated and non-fumigated subsample identification.

6. Evacuate Chloroform from Desiccators Under a Fume Hood

1. Open the stopper on a vacuum desiccator to allow airflow into the desiccator. Remove the lid from the desiccator, and take the samples and the damp towel out of desiccator.
2. Use a vacuum to evacuate $CHCl_3$ gas from soil samples.
    1. Place the lid on the desiccator. Connect the desiccator to a vacuum with vacuum tubing.
    2. Turn on the vacuum pump and allow pump to run for five minutes. Disconnect the vacuum tubing from the desiccator to allow airflow into the desiccator.
    3. Repeat step 6.3.2 four times.

7. Move Each Soil Subsample into an Incubation Container (Figure 1) to Conduct a 10 Day Incubation

1. Pipette 1 ml of deionized water into the incubation container. Connect an empty glass vial to the glass rod extending from the size 10 stopper using a rubber band. The open end of the glass vial should face the base of the stopper. The glass vial should be of a size sufficient to hold up to 40 ml of fluid.
2. Place a vial containing the 30 g soil subsample into the incubation container.
3. Add 1 g of non-fumigated soil from the original soil sample to each of its corresponding subsamples (fumigated and non-fumigated) as inoculum.
4. Pipette 1 ml of 2 M NaOH into the glass vial connected to the stopper/glass rod. Push the stopper/glass rod onto the top of the incubation container. Cover the top of the incubation container with Parafilm.
5. Create an incubation container that contains no soil. Assemble three to five no-soil incubation containers.
    NOTE: The acid used to titrate samples of the no-soil container is essential to the determination of CO_2 mineralization during the incubation period, which is described below in subsection 9.3. As such, multiple no-soil containers are created as a safeguard against incorrect handling or titration of a no-soil incubation container that would create an error in CO_2 mineralization calculation for all samples. The acid used to titrate samples from the no-soil containers should be close in values; a highly dissimilar acid value among the no-soil container samples is likely the result of incorrect sample handling or titration.
    1. Follow the procedures of section 5 to assemble incubation containers.
    2. Follow the procedures of 7.1 and 7.4.

6. Place all incubation containers in a darkened storage area at 25 °C. Leave all incubation containers in the storage area for 10 days.

8. Perform Titration on Each Subsample to Quantify CO_2 Produced by Microbial Respiration during the Incubation Period

1. Remove the glass vial containing the 2 M NaOH from the incubation container.
2. Pipette 2 ml of 1 M $BaCl_2$ into the glass vial containing 2 M NaOH.
3. Add one drop of phenolphthalein ($C_{20}H_{14}O_4$) from a pipette or medicine dropper into the glass vial containing the mixture of $BaCl_2$ and NaOH. Place a magnetic stir bar in the glass vial and place the glass vial on a stir plate.
4. With the stir plate activated, slowly add 0.1 N HCl with a burette until the red coloration of the mixture in the glass vial turns clear.
5. Record the amount of HCl required to change the coloration of the mixture in the glass vial.

9. Determine Microbial Biomass C from Data Collected during the First Fumigation-incubation Cycle[16,21,22]

1. Determine the dry weight of soil in each subsample by multiplying its moist weight by the dry:moist weight ratio obtained in step 3.8.
2. Determine the average amount of HCl used to titrate the no-soil incubation containers.
3. Calculate CO_2 mineralized during the 10-day incubation using the formula:

$$CO2 = (NS - S) \times M \times \left(\frac{E}{W}\right)$$

where CO_2 = CO_2 mineralized during the 10-day incubation
NS = Acid used to titrate samples in no-soil incubation container
S = Acid used to titrate samples that contained soil in the incubation container
M = molarity of the HCl
E = 6, the equivalent weight
W = dry weight of soil contained in the incubation container
4. Calculate microbial biomass C using the formula:

$$BioC = \left(\frac{F - NF}{K}\right)$$

where BioC = microbial biomass C
F = CO_2 mineralized from soil subsamples that were fumigated
NF = CO_2 mineralized from soil subsamples that were non-fumigated
K = fraction of microbial biomass C mineralized to CO_2
   1. Determine the value for K by either direct measurement of ^{14}C mineralization in preliminary tests with the soil or published values[22]. A value of 0.45 is commonly used for K for this assay[23].

5. Perform sequential fumigation and incubation cycles by repeating sections 4-8 seven times for the soil subsamples that were fumigated in the first fumigation-incubation cycle.

10. Determine Labile C and Potential C Turnover Rate Using CO_2 Mineralized over the Course of the Eight Fumigation and Incubation Cycles

1. Use the following formula to determine a correction factor for the soil inoculum added to samples after each fumigation:

$$IC = \left[\left(\frac{C'}{r + 1}\right) + \sum \frac{C_{t-1}}{r + 1}\right]$$

Where IC = Correction factor for inoculum
C' = Amount of CO_2 from the non-fumigated subsample during the first 10-day incubation
r = Weight ratio of inoculum soil to fumigated soil in the first fumigation incubation cycle
C_t = incubation cycle (1, 2 … 8), such that C_{t-1} = 0 when t = 1
2. Use the following formula to estimate the CO_2 released during each incubation for each subsample:

$$Ct = \left[(NS - S) \times M \times E - IC\right]/W$$

where Ct = CO_2 released during incubation
NS = Acid used to titrate samples in no-soil incubation container
S = Acid used to titrate samples that contained soil in the incubation container
IC = Correction factor for inoculum (determined in step 10.1)
E = 6, the equivalent weight
W = dry weight of soil contained in the incubation container
3. Derive labile organic C using non-linear regression.
   1. Organize a spreadsheet that includes for each sample identifiers for the sample, incubation cycle number (1, 2 …8), and CO_2 released during the incubation (derived in step 10.2).
   2. Using software capable of non-linear regression, fit the following model to the dataset:

$$Csum = LOC \times (1 - e^{-kt})$$

where Csum = the sum of CO_2 released during the eight incubation cycles
LOC = soil labile organic C
k = potential turnover time

t = incubation cycle (1, 2 …8)

4. Convert potential turnover time from step 10.3.2 into days by multiplying the inverse of k by 10 due to the 10 day incubation cycle.

Representative Results

The SFI method has been used as described in this paper in a series of experiments conducted in the southeastern United States[24,25,26,27]. Together, these experiments encompassed a variety of vegetation types, including loblolly pine (*Pinus taeda* L.), switchgrass (*Panicum virgatum* L.), cottonwood (*Populus deltoides* Bartram ex Marsh.), and soybean (*Glycine max* L. Merr.). The method was sensitive at determining differences in LOC and/or potential C turnover rates among fertilization and cropping practice treatments in all studies. There was overlap in the range of LOC and potential turnover rates reported in this series of studies (**Figure 2**). Variations in the ranges of LOC reported in these studies highlight the sensitivity of the SFI method in detecting differences in labile C and potential C turnover. The loblolly pine and switchgrass alley cropping system had the greatest range of LOC among the vegetation types; the studies of this vegetation type encompassed the widest array of site conditions relative to the other vegetation types. The sites varied in soil type and age of the loblolly pine overstory from juvenile to late-rotation. The chronosequence of overstory age likely created the largest variation in organic matter inputs among the vegetation types presented here for representative results. The switchgrass pasture vegetation type was studied at the widest array of soil textures, and it also exhibited a relatively high variance in reported values. The soybean vegetation type had relatively high LOC values among site types, which was likely associated with the annual deposition of dead above- and below-ground biomass to the soil during its harvest. Loblolly pine plantations, which are characterized by relatively recalcitrant acidic pine litter as the dominant source of soil organic matter, exhibited the highest potential C turnover rates among the vegetation types selected here for representative results. The range of values reported in this series of studies is also within the range of those found in the range of soils used to develop the SFI method[13] and in later experiments conducted with subtropical forests in China by one of the scientists that developed the SFI method[11,28].

Figure 1. Incubation container for conducting sequential fumigation incubation procedure for labile organic C and potential C turnover rate determination. On the left is the Nalgene bottle, vial suspended from a stopper rod to contain NaOH, and a vial containing 30 g of soil shown separated for demonstration purposes. The container on the right has the soil and the stopper rod positioned within the Nalgene bottle with Parafilm along the top as would be done during incubation. Please click here to view a larger version of this figure.
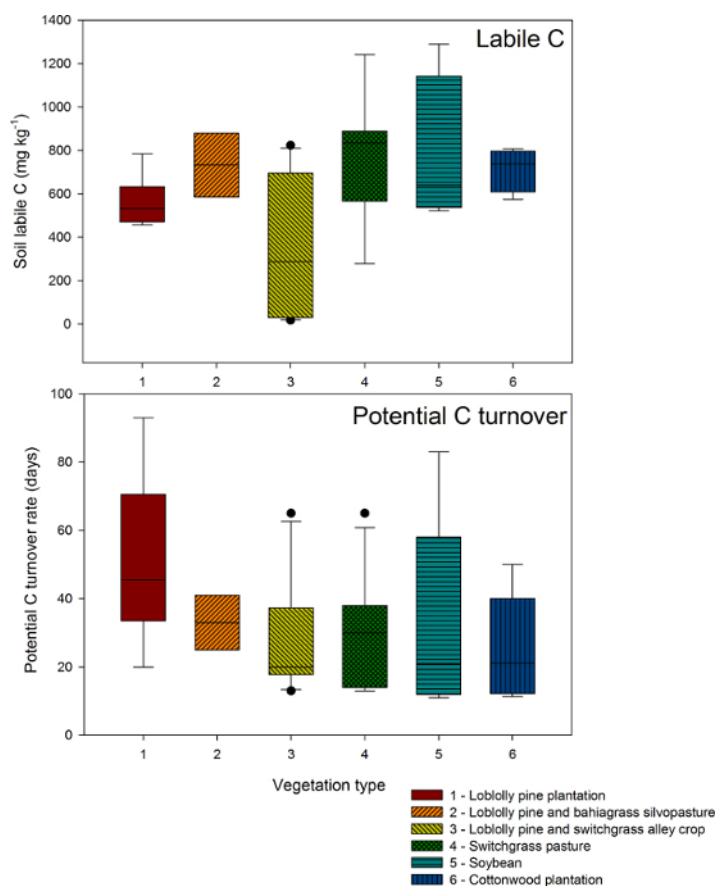
Figure 2. Range of labile organic C and potential C turnover rate as measured with the sequential fumigation incubation method in different soil and vegetation conditions in the southeastern United States. Ranges reported are adapted in part from previous studies[24,25,26,27]. Vegetation types are: (1) loblolly pine plantation, (2) loblolly pine and bahiagrass pasture, (3) loblolly pine and switchgrass alley cropping system, (4) switchgrass pasture, (5) soybean, and (6) cottonwood plantation. Bars represent standard deviation. Please click here to view a larger version of this figure.

Discussion

The SFI method is an effective protocol for detecting differences in soil LOC and potential C turnover rates over a range of management practices (such as fertilization, tillage, vegetation control, and harvest practices) and soil conditions. Soil LOC content and C turnover rate can be used to understand alterations of nutrient cycles. The SFI method also provides measurement of microbial biomass C from the first fumigation-incubation event. The ability to measure soil LOC, C turnover, and microbial biomass C concurrently makes the SFI method a relatively cost-effective analytical method.

Measuring additional soil parameters along with those collected with the SFI method can provide a broader understanding of the impacts of changes in LOC and its associated variables on nutrient cycling. Total C concentration of the soil provides the ability to determine changes in the size of the soil LOC pool relative to total C[29]. Including assays of soil enzymes facilitates understanding of microbial utilization of the labile C substrate that leads to changes in soil nutrient forms and quantities. For example, dehydrogenase plays a key function in organic matter degradation and is a useful indicator of overall microbial actitivity[30]. Urease and phosphatase are examples of enzymes associated with nutrient cycling because they are correlated with microbial transformations of nitrogen and phosphorus, respectively[31,32].

There are variations in procedures of the SFI method, and the number of fumigation and incubation cycles is adjustable to different soil conditions. In the experiment in which the SFI method was developed, five or eight cycles were used for the array of tropical and subtropical forest soils tested[13]. In that experiment, five fumigation-incubation cycles were used for the Oa horizon and deeper mineral soil horizons of an Alfisol soil while eight cycles were used for Ultisol, Oxisol, Inceptisol, and Entisol soils. More recent experiments with the SFI method have used five[28] and seven[11] fumigation-incubation cycles. The key consideration for the number of cycles is whether CO_2 accumulation plateaus over the course of the cycles. The SFI method fails to provide estimates of labile organic C and potential turnover if the negative exponential model does not fit the CO_2 accumulation data. For some soil conditions, such a plateau can be reached within five fumigation-incubation cycles whereas for others more cycles are necessary. Preliminary testing of the SFI method on the soil conditions of interest can aid in determining the appropriate number of cycles necessary. Graphing CO_2 accumulation trends for all samples after each fumigation-incubation cycle can also aid in determining accuracy of the number of cycles to be used.

Due to its dependence on CO_2 accumulation to derive the LOC and C turnover rate, accurate measurements of CO_2 evolution during incubation are essential to the SFI method. The long history associated with the use of the chloroform fumigation-incubation method from which the SFI method was developed has provided a number of recommendations to minimize potential errors. For example, the 10-day pre-incubation

detailed in this SFI protocol description is recommended to allow the influence of soil disturbance to subside and for living fragments of roots to die in order to better isolate microbial CO_2 evolution during the initial incubation period[33,34]. There are several alternatives for incubation container design, but essential components of design include a vial with NaOH to react with CO_2 from microbial respiration, separate containers for the NaOH and soil, a source of moisture to prevent soil from drying during incubation, and seals to prevent contamination from ambient air during the incubation period[22]. Care must also be taken to avoid breathing near the vial of NaOH, which would partially neutralize the alkali and yield erroneous CO_2 measurements, during incubation container assembly[22]. Sample depth in the incubation container can affect accuracy of CO_2 measurements. In a test of soil sample depths within incubation containers, sample depths less than 4 cm had significantly greater inconsistency in CO_2 evolution measurements than depths from 4 to 10 cm[35]. The higher inconsistency from the shallower depths was attributed to greater potential for soil drying during incubation, greater diffusion of O_2 into soil, greater diffusion of CO_2 out of the soil, and a lower potential for anaerobic microorganisms to contribute to decomposition. Soil pH may be another important consideration for the accuracy of CO_2 accumulation measurements. After chloroform fumigation, recolonizing microorganisms during the incubation period are primarily bacterial populations. However, at soil pH values under 4.5, bacterial population development is inhibited. For the chloroform fumigation-incubation method, this effect leads to underestimation of soil microbial biomass C relative to that measured with other methods. Effects of soil pH on values yielded by the SFI method have not yet been tested, but precaution about using the method on low-pH soils may be warranted due to the known effects of pH on chloroform fumigation-incubation results.

The SFI method is one of several methods for measuring LOC, and it is important to distinguish measurements from this method relative to that of others. All LOC methods are an attempt to emulate natural processes of microbial degradation in soil to estimate LOC pool size, but these methods can differ in the portions of the LOC pool they sample and estimate[36]. In a study of labile organic pools in the Wuyi Mountains of China, LOC was measured with $KMnO_4$ oxidation, aqueous extraction, physical fractionation, and the SFI method[37]. Measurements from each method were positively correlated with each other except for light fraction carbon as measured by physical fractionation. However, there were differences in the ranges of values derived from each method that were a function of the different components of the LOC pool each method measured. Generally, the magnitude of LOC values derived from each method was physical fractionation > $KMnO_4$ oxidation > SFI > aqueous extraction. Physical fractionation was done as a measure of light fraction C, which is typically comprised of plant residues and animal remains. However, light fraction C can also contain charcoal and woody debris that decomposes relatively slowly; this LOC pool is an intermediate stage between plant residue and stable organic matter. Labile organic C values from $KMnO_4$ oxidation (termed easily oxidized C) were approximately twice as large as those of the SFI method, which was attributed to the procedure sampling fractions of LOC with slower turnover than the SFI and aqueous extraction methods. Labile organic C measurements from aqueous extraction (termed water-soluble organic C) were approximately ten-fold lower than those of the SFI method. Due to microbial degradation of C being central to the SFI method, its LOC values were interpreted as microbial available C. These microbial available C values were greater than those of microbial biomass C measured in the same experiment, indicating that some of the C remaining in the soil after measuring microbial biomass C was available for mineralization by microbes. Greater values for LOC from the SFI method than those of microbial biomass C are consistent with several previous studies[13,24,25,26,27]. The SFI method has been used in several studies (which represent the majority of reported uses of this method to the authors' knowledge) conducted in an array of locations, vegetation types, and soil conditions[11,13,24,25,26,27,28,38]. Given the apparent differences in the fractions of LOC pools measured by different methods, future applications of the SFI method should be done in concert with LOC methods that estimate different types of labile C as done in the Wuyi Mountain study[36]. Distinguishing LOC measured by the SFI method as microbial available C may be warranted to more precisely describe the type of LOC estimated by SFI. Inferences derived from the SFI method could also be improved by refining understanding of the portion of the LOC pool that is estimated by SFI and its roles in soil organic C and nutrient cycling processes.

Disclosures

The authors have nothing to disclose.

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References

1. Blair, G., *et al.* Soil carbon fractions based on their degree of oxidation, and the development of a carbon management index for agricultural systems. *Aust. J. Agric. Res.*, **46**, 1459-1466 (1995).
2. Schimel, D.S. *et al.* Soil organic matter dynamics in paired rangeland and cropland toposequences in North Dakota. *Geoderma*, **36**, 201-214 (1985).
3. Parton, W.J. *et al.* Analysis of factors controlling soil organic matter levels in great-plains grasslands. *Soil Sci. Soc. Am. J.*, **51**, 1173-1179 (1987).
4. Wu, H. *et al.* Labile organic C and N mineralization of soil aggregate size classes in semiarid grasslands as affected by grazing management. *Biol. Fertil. Soils*, **48**, 305-313 (2011).
5. Jones, D.L *et al.* Plant and mycorrhizal regulation of rhizodeposition. *New Phytol.*, **163**, 459-480 (2004).
6. Harrison, K.G. *et al.* The effect of changing land use of soil radiocarbon. *Science*, **262**, 725-726 (1993).
7. Jinbo, Z. *et al.* Land use effects on the distribution of labile organic carbon fractions through soil profiles *Soil Sci. Soc. Am. J.*, **70**, 660-667 (2006).
8. Whalen, J.K. *et al.* Carbon and nitrogen mineralization from light- and heavy-fraction additions to soil *Soil Biol. Biochem.*, **32**, 1345-1352 (2000).

9. Gregorich, E.G. *et al.* Towards a minimum data set to assess soil organic matter quality in agricultural soils. *Can. J. Soil Sci.*, **74**, 367-385 (1994).
10. Hamer, U. *et al.* Priming effects in different soil types induced by fructose, alanine, oxalic acid and catechol additions. *Soil Biol. Biochem.*, **37**, 445-454 (2005).
11. Feng, W. *et al.* Shifting sources of soil labile organic carbon after termination of plant carbon inputs in a subtropical moist forest of southwest China. *Ecol. Res.*, **26**, 437-444 (2011).
12. Tisdall, J.M. Formation of soil aggregates and accumulation of soil organic matter. In: *Structure and Organic Matter Storage in Agricultural Soils.* Carter, M.R., Stewart, B.A., eds., Lewis Publishers, 57-96 (1996).
13. Zou, X.M. *et al.* Estimating soil labile organic carbon and potential turnover rates using a sequential fumigation-incubation procedure. *Soil Biol. Biochem.*, **37**, 1923-1928 (2005).
14. Cambardella, C.A., Elliott, E.T. Particulate soil organic matter changes across a grassland cultivation sequence. *Soil Sci. Soc. Am. J.*, **56**, 777-783 (1992).
15. Strosser, E. Methods for determination of labile soil organic matter: an overview. *J. Agrobiol.*, **27**, 49-60 (2010).
16. Jenkinson, D.A., Powlson, D.S. The effects of biocidal treatment on metabolism in soil V: a method for measuring soil biomass *Soil Biol.Biochem.*, **8**, 209-213 (1976).
17. Vance, E.D. *et al.* An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.*, **19**, 703-707 (1987).
18. De-Polli, H. *et al.* Chloroform fumigation-extraction labile C pool (microbial biomass C "plus") shows high correlation to microbial biomass C in Argentinian and Brazilian soils. *Cienc. Suelo.*, **25**, 15-22 (2007).
19. Olson, J.S. Energy storage and the balance of producers and decomposers in ecological systems *Ecology.* **44**, 322-331 (1963).
20. Pennock, D. *et al.* Soil sampling designs. In: *Soil Sampling and Methods of Analysis* Carter, M.R., Gregorich, E.G., eds., Chapter 1, Unit 1, CRC Press, Taylor & Francis Group, LLC (2008).
21. Luizao, R.C.C. *et al.* Seasonal variation of soil microbial biomass: the effects of clearfelling a tropical rainforest and establishment of pasture in the central Amazon. *Soil Biol. Biochem.*, **24**, 805-813 (1992).
22. Horwath, W.R., Paul, E.A. *Microbial biomass. In: Methods of soil analysis part 2: microbiological and biochemical properties.* Weaver, R.W. *et al.*, eds., Soil Science Society of America, Inc., 753-773 (1994).
23. Jenkinson, D.S., Ladd, J.N. Microbial biomass in soil: measurement and turnover. In: *Soil Biochemistry.* Paul, E.A., Ladd, J.N., eds., Marcel Dekker, 415-471 (1981).
24. Blazier, M.A. *et al.* Poultry litter fertilization impacts on soil, plant, and water characteristics in loblolly pine (*Pinus taeda* L.) plantations and silvopastures in the mid-South USA. In: *Principles, application, and assessment in soil science*, Gungor, E.B.O., ed., InTech, Inc., 43-74 (2011).
25. Blazier, M.A. *et al.* Straw harvesting, fertilization, and fertilizer type alter soil biophysical properties in a loblolly pine plantation in the mid-South USA. *Biol. Fertil. Soils*, **45**, 145-153 (2008).
26. Blazier, M.A. *et al.* Loblolly pine age and density affects switchgrass growth and soil carbon in an agroforestry system. *For. Sci.*, **58**, 485-496 (2012).
27. Blazier, M.A. *et al.* Nitrogen and carbon of switchgrass, loblolly pine, and cottonwood biofuel production systems in the Southeast United States. *For. Sci.* **61**, 522-534 (2015).
28. Zhang, M. *et al.* Decomposition differences of labile carbon from litter to soil in a tropical rain forest and rubber plantation of Xishuagbanna, southwest China. *Eur. J. Soil Biol.* **55**, 55-61 (2013).
29. Nelson, D.W., Sommers, L.E. Total carbon, organic carbon, and organic matter. In: *Methods of soil analysis. Part 3: chemical methods.* Sparks, D. *et al.*, eds., Soil Science Society of America, Inc., 961-1090 (1996).
30. Huang, L. *et al.* Correlation among soil microorganisms, soil enzyme activities, and removal rates of pollutants in three constructed wetlands purifying micro-polluted river water. *Soil Biol. Biochem.*, **70**, 221-228 (2012).
31. Kong, L. *et al.* Enzyme and root activities in surface-flow constructed wetlands. *Chemosphere*, **76**, 601-608 (2009).
32. Cui, L. *et al.* Evaluation of nutrient removal efficiency and microbial enzyme activity in a baffled subsurface-flow constructed wetland system. *Bioresour. Technol.*, **146**, 656-662 (2013).
33. Jenkinson, D.S. Determination of microbial biomass carbon and nitrogen in soil. In: *Advances in nitrogen cycling in agricultural ecosystems.* Wilson, J.R., ed., CAB International, 368-386 (1988).
34. Sparling, G.P. *et al.* Interference from plant roots in the estimation of soil microbial ATP, C, N, and P. *Soil Biol. Biochem.*, **17**, 275-278 (1985).
35. Christie, P., Beatte, J.A.M. Significance of sample size in measurement of soil microbial biomass by the chloroform fumigation-incubation method. *Soil Biol. Biochem.*, **19**, 149-152 (1987).
36. McLaughlin, K.K., Hobbie, S.E. Comparison of labile soil organic matter fractionation techniques. *Soil Sci. Soc. Am. J.*, **68**, 1616-1625 (2004).
37. Xia, X. *et al.* Variation of soil labile organic carbon pools along an elevational gradient in the Wuyi Mountains, China. *J. Resour. Ecol.*, **1**, 368-374 (2010).