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Estimating soil labile organic carbon and potential turnover rates using a sequential fumigation–incubation procedure

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Abstract

Labile carbon is the fraction of soil organic carbon with most rapid turnover times and its oxidation drives the flux of CO_2 between soils and atmosphere. Available chemical and physical fractionation methods for estimating soil labile organic carbon are indirect and lack a clear biological definition. We have modified the well-established Jenkinson and Powlson's fumigation–incubation technique to estimate soil labile organic carbon using a sequential fumigation–incubation procedure. We define soil labile organic carbon as the fraction of soil organic carbon degradable during microbial growth, assuming that labile organic carbon oxidizes according to a simple negative exponential model. We used five mineral soils and a forest Oa horizon to represent a wide range of organic carbon levels. Soil labile organic carbon varied from 0.8 mg/g in an Entisol to 17.3 mg/g in the Oa materials. Potential turnover time ranged from 24 days in an Alfisol to 102 days in an Ultisol. Soil labile organic carbon contributed from 4.8% in the Alfisol to 11.1% in the Ultisol to the total organic carbon. This new procedure is a relatively easy and simple method for obtaining indices for both the pool sizes and potential turnover rates of soil labile organic carbon and provides a new approach to studying soil organic carbon.
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Keywords: Carbon pools; Carbon turnover rates; Labile organic carbon; Soil carbon; Soil analytical methods

1. Introduction

A gradually increasing flux of CO_2 from soil to the atmosphere may be accelerating global climate warming. Soil labile organic carbon is the most active fraction of soil organic carbon with rapid turnover rates, and it changes substantially after disturbance and management (Coleman et al., 1996; Harrison et al., 1993). Parton et al. (1987) defined soil labile carbon as the fraction of soil organic carbon with a turnover time of less than a few years as compared to recalcitrant carbon with a turnover time of several thousand years. Soil labile organic carbon has been represented operationally by specific fractions derived using chemical and physical separation techniques. Chemical fractionation relies on the solubility of organic carbon in oxidizer, acid or base, producing soluble organic carbon (e.g. Tirol-Padre and Ladha, 2004). Although water and acid hydrolysis (6 M HCl) extractions are recommended for identifying rapid and slow turnover carbon, respectively (Paul et al., 1997), these data fail to identify the chemical constituents and their biological properties (Duxbury et al., 1989). Physical fractionation methods separate soil organic carbon into components as heavy and light carbon (Sollins et al., 1983; Tisdall, 1996) or as coarse and fine particulate organic carbon (Cambardella and Elliott, 1992, 1993). However, these physical fractionation methods do not produce consistent results because data vary with the mineral composition and density found in different soil types, with the size and density that differ among plant materials, and with soil aggregate consistency that fluctuates with dispersibility and particle bounding properties (Sollins et al., 1999). Estimates of light and coarse particulate organic carbon can only give qualitative information about labile organic carbon. Furthermore, estimates of organic carbon pools derived from the existing chemical or physical

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fractionation methods do not provide information on their potential turnover rates, unless one uses tracers such as ^{14}C (Jenkinson et al., 1987; Christensen and Christensen, 1995) or provides estimates of microbial growth rates (Harris and Paul, 1994).

Jenkinson and Powlson (1976) developed a fumigation–incubation procedure to estimate soil microbial carbon. This method was later modified into a fumigation–extraction technique (Sparling and West, 1988; Voroney et al., 1993). Jenkinson et al. (1987) further developed a model that predicts soil carbon fractions and their turnover time using ^{14}C generated data from long-term experiments at Rothamsted. However, these microbial and model estimates have not provided direct measurements on the pool sizes and potential turnover rates of soil labile organic carbon.

Although soil labile organic carbon is constituted of amino acids, simple carbohydrates, a fraction of microbial biomass, and other simple organic compounds, a clear chemical or physical definition of soil labile organic carbon is difficult if not impossible. We here present a biological definition of soil labile organic carbon as microbial degradable carbon associated with microbial growth. This biological definition includes two aspects: soil labile organic carbon is both chemically degradable and physically accessible by soil microbes. Organic carbon that is chemically degradable but physically inaccessible by microbes due to clay mineral protection is not regarded here as soil labile organic carbon.

The objective of this study is to develop a new method for estimating indices of biologically-defined soil labile carbon pools and their potential turnover rates. We adopted Jenkinson and Powlson's fumigation–incubation approach for estimating soil labile carbon as the accumulated CO_2 released from microbial growth using a sequential fumigation–incubation procedure. We assume that soil labile carbon is degraded according to the simple negative exponential equation as being widely seen in plant litter decomposition (Olson, 1963). The accumulated microbial carbon from the sequential fumigation–incubation can then be estimated using a simple first-order kinetics model (Stanford and Smith, 1972). Relying on the established fumigation–incubation procedure which is relatively simple in operation, this method provides indices of both labile organic carbon pools and their potential turnover rates. One can obtain indices of labile organic carbon pools and potential turnover rates within a period of less than 3 months.

2. Materials and methods

2.1. Soils

We used five mineral soils and an Oa forest-floor material representing a wide range of carbon levels. These soils were obtained from tropical forests in Puerto Rico and a subtropical evergreen-broad-leaved forest in Yunnan of China. One Inceptisol was from an alluvial deposit by a stream bank in tabonuco forest (Zou et al., 1995) that was dominated by *Dacryodes excelsa* Vahl at El Verde Field Station in the Luquillo National Forest (10°20′N, 65°49′W), Puerto Rico. An Entisol was obtained from landslide scars within the tabonuco forest where parent materials were exposed. An Oxisol was a well-weathered tropical soil within the tabonuco forest from upland areas. These three soils were located at an elevation of about 450 m above the sea level, where the monthly temperature ranges between 20.8 and 14.4 °C with an annual average precipitation of 3467 mm (Soil Survey Staff, 1995). An Ultisol was obtained from the cloud forest of the Luquillo Mountains at an elevation of about 950 m above the sea level. The cloud forest was dominated by *Tabebuia rigida* (Walker et al., 1996). Annual mean temperature ranged from 17 to 20 °C with the annual precipitation reaching 4200 mm (Brown et al., 1983). An Alfisol and its Oa layer were obtained from the Ailaoshan Nature Reserve of the Yunnan province, southwestern China (24°30′N, 101°00′W). The subtropical evergreen-broad-leaved forest was dominated by *Castanopsis watii*, *Lithocarpus chingdongensis*, *Lithocarpus xylocarpus*, and *Machilus veridis* (Young et al., 1992) with annual mean temperature of 10.8 °C and annual mean precipitation of 1900 mm (Liu, 1993).

Each soil was sampled to a depth of 100 mm with five soil cores which were bulked into a composite sample. We did not sieve the soil, but plant roots >2 mm in diameter, rocks, and soil macrofauna were removed. The floor mass Oa layer was obtained by passing floor mass through a 3 mm sieve. Soils were thoroughly homogenized by hand kneading before analyses. Soil total carbon and N were determined by combustion in a LECO 2000 CHN elemental analyzer (LECO Corporation, St Joseph, MI, USA). Element concentrations of P, K, Ca, Mg, Fe, and Al were obtained using a Beckman DCP spectraspan V (Beckman Instruments, Fullerton, CA, USA) after samples had been digested with H_2O_2 and concentrated HNO_3 (Parkinson and Allen, 1975).

2.2. Microbial biomass and labile organic carbon

We used the modified Jenkinson and Powlson's fumigation–incubation method (Liu and Zou, 2002) for estimating microbial biomass. A subsample of 10 g of soil was oven-dried at 105 °C for 24 h for determining soil moisture. Two subsamples of 30 g of soil (wet weight) each were used for the fumigation and control treatments. Soil microbial biomass was calculated from difference in CO_2 evolved during the first 10 days' incubation from the fumigated and control soils. Microbial biomass was calculated as (Jenkinson and Powlson, 1976): $B=F/K$, where B is soil biomass carbon in mg C/g soil; F is CO_2–C evolved during the 10 days' incubation from the fumigated soil, less that evolved from control soil incubated for the same time under the same conditions; and $K=0.45$, or

Table 1
Chemical properties of soils used for the sequential fumigation–incubation study

Soil order	C (%; ±SE)	N (%; ±SE)	P (mg/g) (±SE)	K (mg/g) (±SE)	Ca (mg/g) (±SE)	Mg (mg/g) (±SE)	Fe (mg/g) (±SE)	Al (mg/g) (±SE)
Entisol	1.27 (0.60)	0.08 (0.04)	0.71 (0.21)	1.33 (0.14)	0.14 (0.05)	0.74 (0.22)	73.38 (4.84)	47.67 (6.89)
Inceptisol	2.71 (0.25)	0.23 (0.03)	0.29 (0.02)	1.28 (0.05)	2.00 (0.23)	6.64 (1.07)	72.33 (4.23)	42.92 (2.66)
Alfisol	11.23 (0.61)	0.63 (0.05)	0.81 (0.04)	9.99 (0.71)	0.62 (0.05)	3.12 (0.16)	42.99 (1.59)	55.22 (3.65)
Ultisol	11.87 (2.70)	0.52 (0.10)	0.15 (0.05)	1.10 (0.21)	0.37 (0.11)	0.44 (0.04)	63.10 (13.25)	31.57 (5.53)
Oxisol	4.98 (0.35)	0.41 (0.02)	0.26 (0.01)	1.09 (0.11)	0.60 (0.23)	2.52 (0.72)	74.42 (2.23)	52.06 (5.90)
Oa[a]	34.24	0.81	0.91	3.01	4.10	1.21	11.80	11.79

[a] Note: Data from Liu et al. (1995).

the fraction of the biomass carbon mineralization to CO_2–C following the fumigation–incubation. To estimate soil labile organic carbon, the fumigated subsamples were subjected to the fumigation–incubation procedure for a total of 5–8 cycles (n, the number of fumigation–incubation cycles). Carbon dioxide evolved from the first and the subsequent incubations of the fumigated soils was used for estimating labile organic carbon, after adjusting for the addition of 1 g inoculation soil. The evolved soil CO_2 carbon (C_t in milligrams per gram of soil) following the tth fumigation–incubation cycle was calculated using the modified formula of Carter (1993): $C_t=[(A_t-V_t)N_tE-Q_t]/w$, where A_t is the volume (milliliters) of acid needed to titrate the NaOH in the beakers for the blank; V_t is the volume (milliliters) of acid needed to titrate the NaOH in the beakers containing the fumigated soils; N_t is the normality of the acid; $E=6$, the equivalent weight; and w is the initial weight of soil for fumigation treatment. $Q_t=C'/(r+1)+\Sigma[C_{t-1}/(r+1)]$, $t=1,\ldots, n$, is a correction factor for the inoculated soil after each fumigation, where $C_{t-1}=0$ when $t=1$; C' is the amount of CO_2–C from the control soil during the first 10-day incubation; and r is the weight ratio of fumigated soil to inoculation soil. The accumulated CO_2–C (M_t, $t=1,\ldots,n$) from the fumigated soil is calculated according to Stanford and Smith (1972)

$$M_t = C_{labile}(1 - e^{-kt}); \quad (1)$$

where C_{labile} is the estimated pool size of soil labile organic carbon and k is the potential turnover rate. Potential turnover time (days) of soil labile organic carbon is calculated as the inverse of k multiplied by 10 because each incubation period is 10 days in length. Values for C_{labile} and k can be estimated using linear regression with the following transformed equation:

$$Ln(C_t) = Ln(kC_{labile}) - kt, (t = 1, 2, \ldots, n); \quad (2)$$

where k is the slope, Ln (kC_{labile}) is the intercept (a), and $C_{labile}=e^a/k$.

3. Results

Soil total organic carbon varied across soil types with the lowest carbon content of 1.3% occurring in the landslide Entisol and highest value of 11.9% in the Ultisol from the cloud forest (Table 1). Because of the inclusion of a fraction of mineral soil, the Oa materials had a total organic carbon concentration of 34.2%. Soil total nitrogen concentrations were the lowest in the Entisol with a value of 0.08% and highest in the Alfisol with a value reaching 0.63%. However, the concentrations of the divalent cations Ca and Mg were highest in the river alluvial Inceptisol. Concentrations of P and K were higher in the Alfisol than in other soils. The Oxisol had the highest values for Fe and the Alfisol the highest value for Al, indicating high levels of acidity.

Soil microbial biomass carbon levels were highest in the Alfisol (2.5 mg/g) and its Oa (4.2 mg/g) materials from the evergreen-broad-leaved forest, and lowest in the landslide Entisol with a value of 0.20 mg/g of soil (Table 2). Microbial biomass carbon in the Oxisol was 1.36 mg/g of soil, similar to the 1.30 mg/g (Ruan et al., 2004) or 1.50 mg/g (Liu and Zou, 2002) measured previously in the same forest, but higher than the 0.7 mg/g from the adjacent pine plantation soil (Li et al., in press). Correlations between Ln-transformed values of CO_2–C evolved from the fumigated samples and the sequential fumigation–incubation cycles (t) were all linear (Eq. (2)) and significant (Fig. 1). Estimates of soil labile organic carbon pools ranged from 0.80 mg/g in the landslide Entisol to 13.17 mg/g in the Ultisol from the cloud forest with a 165-fold difference. Labile organic carbon reached 17.27 mg/g in the Oa layer. In contrast, the potential turnover rates (k) of soil labile organic carbon were fastest in the Alfisol and slowest in the Ultisol with the potential turnover time of 24 and 102 days, respectively.

Table 2
Pool sizes and potential turnover rates (k) of soil labile organic C in various soils as estimated by the sequential fumigation–incubation procedure

Soil order	$C_{Microbial}$ (mg/g)	C_{Labile} (mg/g)	k (cycles^{-1})	$10/k$ (day)	$C_{Microbial}/C_{Labile}$ (%)	C_{Labile}/C_{Total} (%)
Entisol	0.20	0.80	0.278	36.0	25.25	6.27
Inceptisol	0.73	1.89	0.256	39.1	38.55	6.99
Alfisol	2.51	5.39	0.420	23.8	46.49	4.80
Ultisol	1.52	13.15	0.098	102.2	11.57	11.07
Oxisol	1.37	3.82	0.188	53.2	35.81	7.67
Oa	4.21	17.27	0.308	32.5	24.35	5.04

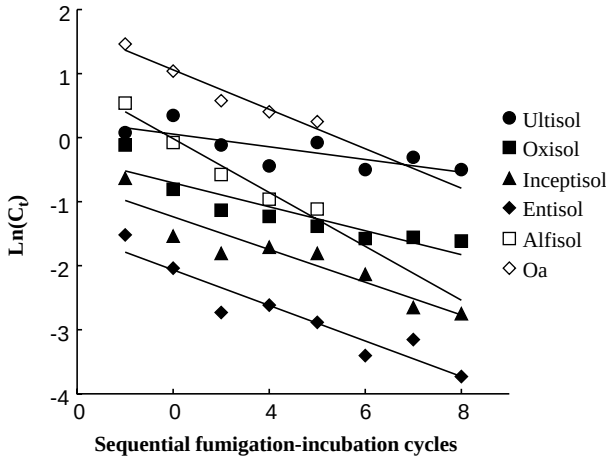

Fig. 1. Linear correlation between Ln-transformed values of CO_2–C (mg/g) released from the corresponding sequential fumigation–incubation treatment cycles for five tropical soils and a floor mass Oa layer. The slopes and intercepts in these correlations were used to estimate soil labile organic carbon pools and their potential turnover rates.

The accumulated CO_2–C evolved from the sequential fumigation–incubation fitted well with Eq. (1) when plotted against the sequential fumigation–incubation treatment cycles (Fig. 2). Eight round of fumigation–incubation treatments released over 50% of the estimated soil labile organic carbon pools for all soils, reaching the highest value of 90% in the Entisol. The fraction of soil microbial biomass carbon to the labile organic carbon pool was highest in the Alfisol and lowest in the Ultisol with values of 46.5 and 11.6%, respectively. Labile organic carbon contributed the largest proportion to total soil organic carbon for the Ultisol soil, reaching 11.1%. This proportion varied between 4.8 and 7.7% for other soil samples.

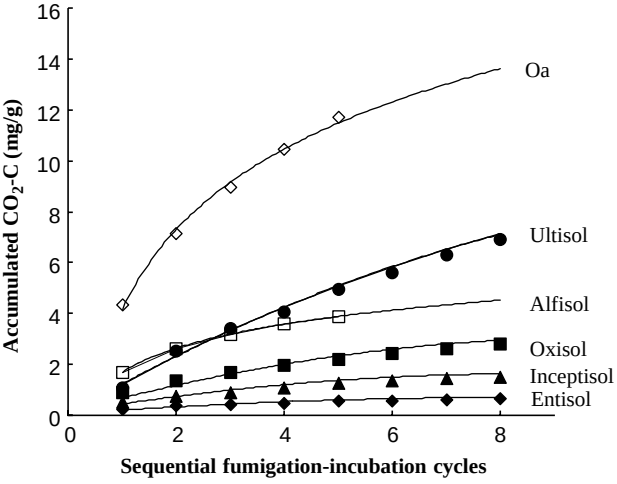

Fig. 2. Accumulated CO_2–C (M_t, mg/g) released from the sequential fumigation–incubation cycles (t) as fitted to the equation $M_t = C_{labile}(1-e^{-kt})$, where C_{labile} is the estimated pool size of soil labile organic carbon, and k is the potential turnover rate.

4. Discussion

Available information on soil labile organic carbon is scarce and inconsistent because of the lacking of a clear biological definition of labile organic carbon and available analytical methods. Microbial carbon contributes 1–3% to soil total organic carbon and was suggested as an index for soil labile organic carbon (Paul and Clark, 1996). Our estimates of soil microbial carbon from five tropical soils all fall within this range. Although microbial biomass carbon levels were similar between the Ultisol and Oxisol soils, the Oxisol (2.7%) had a much higher percentage of microbial carbon to total organic carbon than the Ultisol (1.3%). In contrast, the percentage of the estimated labile organic carbon to soil total organic carbon reached 11.1% in the Ultisol, much higher than 7.6% in the Oxisol. These contrasting data suggested a poor positive correlation between soil microbial biomass carbon and labile organic carbon.

We assumed that the decay of soil labile organic carbon follows an empirical simple negative exponential model as is widely accepted in plant litter decomposition (Olson, 1963). Our data showed a well fit to this model, suggesting that there was no severe violation of this assumption. An inherent assumption that this new method to estimate labile organic carbon had in common with Jenkinson and Powlson's fumigation–incubation procedure was that the fumigation treatment did not change the labile nature of soil organic carbon (Smith et al., 1995; Horwath et al., 1996).

Our data indicated that young soils such as Inceptisols and Entisols had lower levels of microbial biomass carbon and labile organic carbon than older soils such as Ultisols and Oxisols. However, these young soils did not differ from the older soils in the proportion of microbial biomass carbon to total organic carbon. Our data also indicated that organic carbon levels in young soils were more labile than those in older soils with higher potential turnover rates and shorter potential turnover time for the young soils, suggesting that older soils either contain high proportion of old organic materials or have high levels of organo-mineral association. We introduced the concepts of potential turnover rate and potential turnover time because our measurements were derived from microbial growth conditions altered from those in the field. Microbial growth can be limited by soil available pore space, carbon and nutrient availability, or predation activities under field conditions. Fumigation eliminates these constraints, creating a relatively ideal condition for microbial growth. Because the sequential fumigation–incubation procedure does not remove nutrients from soil and eliminates predation, the subsequent microbial growth is largely limited by carbon availability both in total quantity and its relative lability or quality as reflected by the potential turnover rate k. The introduction of potential turnover rate for describing labile organic carbon can be proven to be an important parameter in the future studies of carbon cycling

associated with natural and human disturbance and global climate changes.

This new method also provides a means for the determination of soil recalcitrant organic carbon or slow turnover organic carbon as the difference between total organic carbon and labile organic carbon. Unlike labile organic carbon, this method does not provide information on the quality of recalcitrant organic carbon due to the inability to obtain its potential turnover rates. One may use isotopic labeled materials to obtain this information. This sequential fumigation–incubation procedure is based on the widely established Jenkinson and Powlson's fumigation–incubation technique. It is inexpensive, relatively simple in operation, and provides indices on both pool sizes of labile organic carbon and their potential turnover rates (a parameter for the quality of labile organic carbon available to soil microbes). Compared with the long-term incubation of soil CO_2 evolution for several years or even decades, this new method can obtain both pool sizes and potential turnover rates of soil labile organic carbon within a period of 3 months.

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