E-International Scientific Research Journal
ISSN: 2094-1749 Volume: 3 Issue: 2, 2011

Short-Term Dynamics of the Active and Passive Soil Organic Carbon Pools in a Volcanic Soil Treated With Fresh Organic Matter

Wilfredo A. Dumale, Jr.[1, 2, *], Tsuyoshi Miyazaki [2], Taku Nishimura [2] and Katsutoshi Seki[3]

[1] Department of Plant Science, Nueva Vizcaya State University,
Bayombong 3700, Nueva Vizcaya, Philippines
[2] Department of Biological and Environmental Engineering, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657 Japan
[3] Faculty of Business Administration, Toyo University, 5-28-20
Hakusan, Bunkyo-ku, Tokyo 112-8606, Japan

* Corresponding author,
e-mail: dumalewajr@soil.en.a.u-tokyo.ac.jp; dumalewajr@nvsu.edu.ph

Abstract

In a 110-day constant temperature experiment (20° C), we determined the effect of fresh organic matters (FOM): 0 (control); 1.81 g leaf litter (LL) carbon kg^{-1}; and 2.12 g chicken manure (CM) carbon kg^{-1} in the stable soil organic carbon [mineral-associated organic carbon (MAOC)], labile soil organic carbon [soil microbial biomass carbon (SMBC)], and carbon dioxide (CO_2) evolution of a volcanic ash soil from Tsumagoi, Gunma Prefecture, Japan (138°30' E, 36°30' N).

Overall, CO_2 evolution and SMBC increased after the treatment of soil with FOM, whereas MAOC decreased below its original level three days after FOM application. These data support the view that fresh OM promotes increases in SMBC and CO_2 in the rapidly cycling active carbon pool and further suggest that the MAOC fraction, though stable as conventionally believed, can be a source of CO_2. Our findings challenge the convention that only labile SOC is the source of short-term CO_2 evolution from soils.

Keywords: mineral-associated organic carbon, soil microbial biomass carbon, soil organic carbon, CO_2 evolution

Introduction

Soil organic carbon (SOC) is the largest pool within the terrestrial carbon cycle (Gerzabek *et al.*, 2001), consisting of a heterogeneous mixture of organic matter originating from plant, microbial and animal residues (Baldock and Skjemstad, 2000). A variety of terrestrial ecosystem models have been developed recently to study the impacts of management and/or climate change on SOC turnover under different climates, topographies and management (Sherrod *et al.*, 2005). For example, the CENTURY model is a terrestrial SOC model which partitions SOC into three

conceptual pools: active, slow, and passive, which differ in turnover times (Parton *et al.*, 1988). The relationships of the measurable fractions of these conceptual pools and their measurable fractions with the particle size fractions were summarized by Dumale *et al.* (2009) (Table 1). The mineral-associated organic carbon (MAOC) is the measurable fraction of the passive SOC pool (Sherrod *et al.*, 2005). The MAOC fraction can be measured by physically separating the <53 mm particle size fraction, which is the silt-and clay-sized fraction (Haile-Mariam *et al.*, 2008). The associated SOC of the combined silt and clay is the MAOC (Cambardella and Elliot, 1992).

Table 1. Matrix table indicating relationships of conceptual SOM pools, their measurable fractions, and particle size fractions (Dumale *et al.*, 2009)

Description	Conceptual SOM pools**		
	Active	Slow	Passive
1.Turn-over time	hours to months; 2- to 4-years	Decadal; 20- to 50-years	centuries to millennia; 800–2000 years
2.Representative SOM Fraction***	SMBC (soil microbial biomass carbon)	POMC (particulate organic matter carbon)	MAOC (mineral-associated organic carbon)
3.Description of the fraction	active soil organic matter (SOM) consisting of live microbes and microbial products	protected fraction that is more resistant to decomposition	physically-protected or chemically resistant and has long turnover time
4.Chemical composition	chloroform-labile, microwave-irradiation-labile SOM, amino compounds, phospholipids	amino compounds; glycoproteins; aggregate protected POM; acid/base hydrolyzable; mobile humic acids	aliphatic macromolecules; charcoal; sporopolleins; lignins; high molecular, condensed SOM, humin, nonhydrolyzable SOM, fine silt, coarse-clay associated SOM
5.SOM fraction association with soil particle sizes	Fumigated and extracted SMBC	2mm–53μm; Sand-sized or larger	<53 μm, silt and clay-sized**** referred to as MAOC in this paper

****** The term "pool" is used to refer to the theoretically separated, kinetically delineated components of SOM
******* The term "fraction" is used to describe measurable organic matter components associated with the pool
******** Silt and clay-sized particles were <53 μm diameter based on the USDA Soil Texture Classification System

The particle size fractions play different roles in stabilization of soil organic matter (SOM). The major part of the SOM is usually associated with the clay- and silt-sized fractions (Ohm *et al.*, 2007). Fine-textured soils have higher organic C and N contents than coarse-textured soils when supplied with similar input of organic material (Hassink, 1997). It was assumed that the difference was due to the ability of fine-textured soils to provide greater protection to soil organic matter (Hassink, 1997; van Veen and Kuikman, 1990), and physical protection of SOM is due to its ability to associate with clay and silt particles (Li *et al.*, 2007; Zhao *et al.*, 2006). The SOM associated with silt- and clay-sized fractions is often older than in the sand fractions, which

is attributed to the stabilization mechanisms through surface interactions (Baldock and Skjemstad, 2000; Lützow *et al.*, 2006; Rumpel *et al.*, 2002). The silt- and clay-associated C was older in the light fraction (LF) and particulate organic matter (POM) (Haile-Mariam *et al.*, 2008). Further, the clay-associated residues have the highest mean residence times (MRT).

Most of the input of carbon to soil from different sources is subject to microbial attack, explaining the extra CO_2 mineralization soon after addition to soil. A part, however, are retained and stabilized into the soil over long period of time. Previously, it was suggested that this extra CO_2 originates from the labile SOC fraction. However, from more recent studies, it seems unlikely that only the labile pool is affected, since it cannot fully account for the extra CO_2 released (Hamer and Marschner, 2005). The extra CO_2 evolution can originate from the various pools of SOM (Kuzyakov, 2006). Some studies have found that organic matter (OM) application does not increase SOC (Foereid *et al.*, 2004; Fontaine *et al.*, 2004; Fontaine *et al.*, 2003; Bell *et al.*, 2003; Campbell *et al.*, 1991). Others have reported gains in SOC after years of OM addition to soil (Gerzabek *et al.*, 2001; Gerzabek *et al.*, 1997; Dalenberg and Jager, 1989).

We separated the soil microbial biomass carbon (SMBC) as a measure of the labile soil organic carbon using a modification of the fumigation extraction technique (Vance *et al.*, 1987) and the mineral-associated organic carbon (MAOC) fraction as a measure of the stable soil organic carbon using combined chemical dispersion and physical fractionation (Sherrod *et al.*, 2005; Haile-Mariam *et al.*, 2008; Cambardella and Elliot, 1992).

Our objectives are to (1) determine the short–term influence of fresh organic matter (FOM) application on the dynamics of MAOC, and (2) study the dynamics of SMBC and CO_2 evolution in soils applied with fresh organic matters. We hypothesized that although the MAOC is stable soil organic carbon due to physical protection in the silt and clay fractions, it does contribute to C turnover in the short-term, although conventionally believed to turn over in centuries to millennial time scales.

Materials and Methods

Soil sampling and FOM preparation

Soil samples collected from the 0–5- and 6–20-cm layers of an upland field located in Tsumagoi, Gunma Prefecture, Japan (138°30’ E, 36°30’ N) were air-dried in the shade, sieved through a 2-mm mesh screen, and stored at 4°C until experimentation. Some of the physico-chemical properties of the soil are presented in Table 2. Most of the plant residue was removed by flotation, followed by drying of the soil. Leaf litter (362.7 g kg^{-1} C; 18.0 g kg^{-1} N; 20.1 C/N ratio) and chicken manure (424.9 g kg^{-1} C; 52.5 g kg^{-1} N; 8.1 C/N ratio) were used as FOM. The FOM was air-dried for one week in the shade and then finely ground and passed through a 0.5-mm mesh screen. FOM was stored at 4°C until use.

Table 2. Some physico-chemical properties of the Tsumagoi soil, Gunma Prefecture, Japan.

Depth (cm)	Soil texture	Particle density (gcm^{-3})	Bulk density (gcm^{-3})	Total C (gkg^{-1})	Total N (gkg^{-1})	C/N ratio	Land use/ Common vegetation
0–5	sandy loam	2.48	0.44	70.57	4.76	14.83	Agricultural experimental field; cabbage
5–20		2.48	0.5	88.9	5.7	15.6	

Incubation experiment

Transparent 500-mL glass bottles with plastic lid were used for incubation. (Figure 1). Three holes, one 12.5-mm diameter and two 10-mm diameter, were bored on the lid in triangular fashion. A "cock-rubber stopper" assembly, inserted into the 10-mm holes, served dually as air outlet of "old air" inside the bottles and air inlet of "new moist air" after every sampling day. This "cock-rubber stopper" assembly was made by inserting a three-way plastic cock (Top Corp., Japan) into a 14 x 15.5 x 10.5 mm rubber stopper.

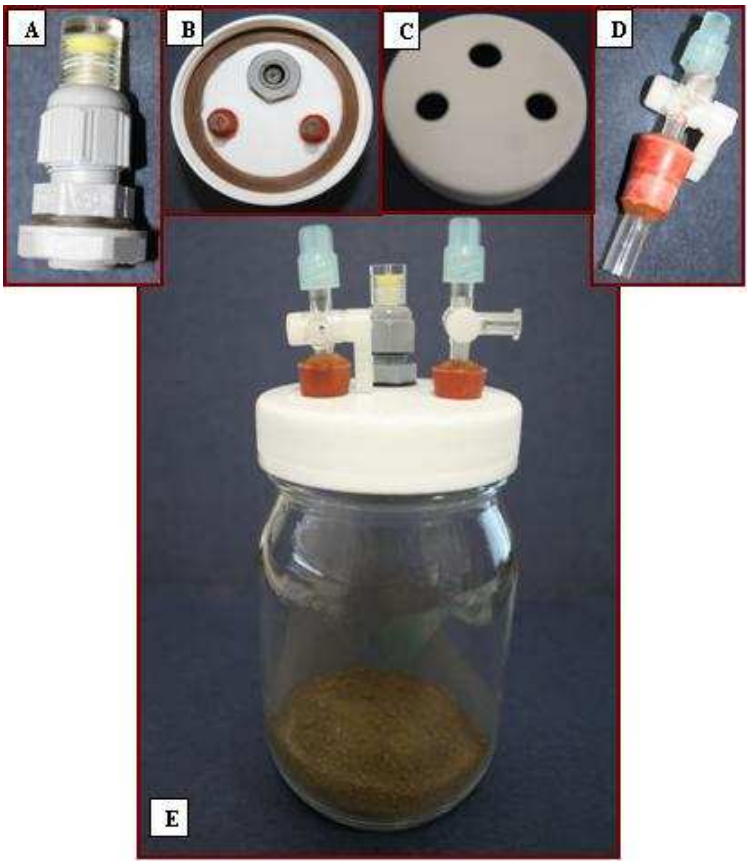

Figure 1. The experimental unit. Acrylic tubing fitted with a septum mounted on cable grand served as the gas sampling port (A); Viewed from the bottom of the lid are the gas sampling port,

inlet and outlet "cock-rubber stopper" assembly (B); the triangular boring in the bottle lid (C); the "cock-rubber stopper" assembly (D); and the assembled experimental unit (E).

Also, a self-designed 35-mm length acrylic tubing sealed with a rubber septum was fitted in the 12.5-mm diameter hole in the bottle lid through a cable grand. This tubing served as the gas sampling port for CO_2 evolution measurement. All assembled incubation bottles were tested leak-free by immersing in a pail of water.

Each experimental unit consisted of 20-g soil samples adjusted to 50% of the soil's water-holding capacity. Incubation was conducted for 110 days at 20°C constant temperature. Prior to sealing each incubation bottle, FOM was evenly incorporated to the soil according to treatment rates. Experimental units allowed for three replicates per treatment on each sampling day. Parameters were measured by destructive sampling at 3, 13, 21, 44, 70, 85, and 110 days after FOM application. For MAOC, measurement was also conducted at day zero.

Separation and measurement of the MAOC fraction

Combined chemical dispersion and particle size separation methods based on the work of several authors (Sherrod *et al.*, 2005; Haile-Mariam, *et al.*, 2008; Cambardella and Elliot, 1992; Bell *et al.*, 2003) were used to separate the combined silt- and clay-sized fractions which contain the MAOC.

On each sampling day, 5-g subsample was placed in 100-mL plastic bottle and dispersed with 50 mL of sodium hexametaphosphate (5 g/L). The suspension was shaken in a reciprocating shaker (Yamato shaker model SA-31, Yamato Scientific Co., Ltd., Japan) overnight at 240 rpm. The soil suspensions were sieved in a 53-µm screen (Tokyo Screen Co. Ltd., Japan). During sieving, the particles retained in the screen were repeatedly rinsed with distilled water to ensure thorough separation of the <53 µm particle size fraction.

The resulting suspension was dried overnight at 70° C. The dried samples were finely ground manually using mortar and pestle and passed through an 80-µm sieve (Tokyo Screen Co. Ltd., Japan). MAOC was measured by dry combustion using a Sumigraph NC-90A NC analyzer (Sumika Inc., Japan).

Gas sampling and CO_2 evolution measurement

Gas samples for CO_2 evolution measurement were drawn from incubation bottles using a 10-mL plastic syringe (Nipro, Japan) fitted with 0.70 x 38.00 mm needle (Nipro, Japan). Transparent 7-mL capacity glass vials were used as sample containers. Prior to sampling, the vials were vacuumed by subjecting to 2 millibars suction for about 10 min and sealed with a rubber septum.

Prior to drawing gas samples, the air inside each incubation bottle was homogenized by alternate pumping and sucking using the sampling syringe 4–5 times. Seven mL of gas sample were drawn and injected into the sample vials. From there, 1 mL of gas sample was drawn and injected into a 16A Gas Chromatograph (Shimadzu Inc.). Each sampling day, after drawing gas samples, the air inside the bottles were flashed out and substituted with moist air through the

inlet and outlet cocks mounted in the bottle lid. The inlet cock was connected to an air source passing through a tank of distilled water to moisten the air and maintain moisture inside the incubation bottles. The outlet cock was simultaneously opened while moist air flowed through the inlet cock at 2.5 kgf cm^{-2} for 3 min to ensure flushing out of "old air" from the bottles.

SMBC fumigation, extraction, and measurement

The soil microbial biomass carbon (SMBC) fumigation and extraction technique used was slightly modified from the fumigation-extraction method described by Vance *et al.* (1987). Each sampling day, 5-g subsamples were placed in small Petri dishes and placed inside a glass desiccator containing 40 mL of ethanol-free chloroform ($CHCl_3$) in a small beaker. To enhance vapor production, the beaker of $CHCl_3$ was immersed in a cup of hot water. The desiccator was sealed and placed in the dark at 25°C for 24 h. After 24 h the beaker of $CHCl_3$ was removed, and the residual $CHCl_3$ vapor in the soil was removed by repeated evacuation using a vacuum pump connected to the desiccator.

For extraction, the samples were transferred to 100-mL plastic bottles, diluted with 50 mL of potassium sulfate (0.5 M K_2SO_4), and shaken in an oscillating shaker at 240 rpm. After 30 min, the suspension was filtered using Whatman No. 42 filter paper followed by membrane filtration using 0.2-µm Millex syringe-driven filter units. A separate set of unfumigated samples was also prepared for use as control. The filtered samples were analyzed using a Total Organic Carbon Analyzer (Shimadzu TOC-VCSN, Shimadzu, Inc.). SMBC was calculated using the formula, SMBC = $2.64E_c$, where E_c is the difference between the organic carbon extracted from the fumigated and non-fumigated samples (Vance *et al.*, 1987).

Statistical treatment of data

Data were subjected to statistical analysis following the split-split plot design to compare and determine any significant differences between and among treatment means. The analysis of variance (ANOVA) was done using the SAS software (SAS Institute). Comparisons of means were done using the least significant difference (LSD) or the Duncan's multiple range test (DMRT) where appropriate.

Results and Discussion

CO_2 evolution rate and cumulative CO_2 evolution

The addition of leaf litter and chicken manure increased the CO_2 flux in soil (Figure 2). Soils that received chicken manure had exceptionally higher CO_2 production than did soils that received leaf litter. The peak of CO_2 production occurred during the first three days of incubation, except for soils from the 0–5-cm layer that received chicken manure, which peaked at day 13 of incubation. The 6–20-cm layer that received chicken manure exhibited the highest CO_2 production, reaching as high as 122 mg kg^{-1} day^{-1} during the first three days after OM application. In the 0–5-cm layer, CO_2 production peaked at 74.6 mg kg^{-1} day^{-1} at 3 to 13 days after FOM application. There was little difference in CO_2 production between soils from the 0–5-

cm (0.49) and 6–20-cm (0.52 mg CO_2 kg^{-1} day^{-1}) layers that received leaf litter during the 120-day incubation period.

The carbon equivalent of the total CO_2 produced in the 0–5- and 6–20-cm layers of soil and between the control and leaf litter treatments ranged from 109 to 178 mg kg^{-1} for the 120-day incubation period. For soils that received chicken manure, the carbon equivalent reached as high as 527 and 828 mg carbon kg^{-1} for the 0–5- and 6–20-cm layers, respectively.

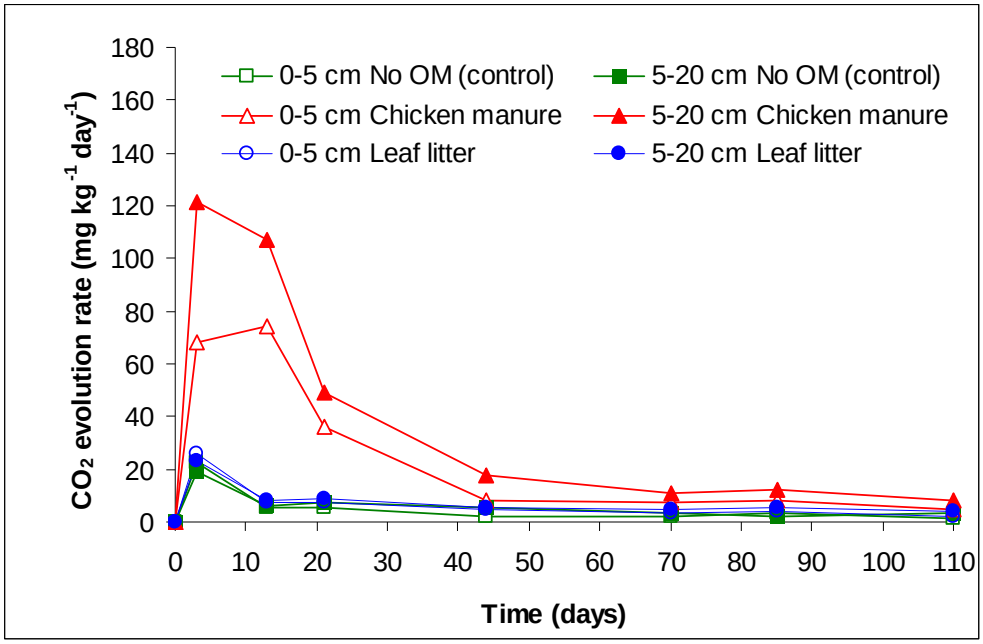

Figure 2. Carbon dioxide evolution rate (mg kg^{-1} day^{-1}) of Tsumagoi soil, Gunma Prefecture, Japan over 110 days of incubation following application of leaf litter and chicken manure.

Soils that received chicken manure produced 199.7% and 531.9% more CO_2 than did controls from the 0–5- and 6–20-cm soil layers, respectively. Approximately, 32–64% of the total evolved CO_2 was released during the first 21 days after FOM addition. In soils that received leaf litter, both the 0–5- and 6–20-cm layers had total evolved CO_2 levels similar to those of the controls. Thus, in contrast to chicken manure, leaf litter has negligible effects on CO_2 production when applied to soil. In the 0–5-cm layer, CO_2 evolution rate in the control was highest 0–3 days after FOM addition (Table 3). Beyond this period, CO_2 evolution rates were statistically lower until the end of incubation. CO_2 evolution rates were comparable during the 4–110-day periods. This is exactly the trend in the leaf litter-applied 0–5-cm layer. In the chicken manure-applied soils, CO_2 evolution rates significantly varied, and highest during the early stage of incubation (0–13 days after FOM addition), when rate was in the range 68.24 to 74.55 mg kg^{-1} day^{-1}. Starting from two weeks after FOM application, CO_2 evolution rate significantly dropped by more than half of the 4–13-day period level, decreasing with time until the end of incubation. From 14–110 days after FOM addition, evolution rates did not significantly differ.

In the 0–5-cm layer without manure (control), CO_2 evolution rate was in the range 5.43 to 19.26 mg $kg^{-1}day^{-1}$ during the 0–44-day period, but from 4–110 days after FOM application, rates were

also statistically the same. In the leaf litter-applied soils, CO_2 evolution rate during the 0–3-day period (23.03 mg $kg^{-1}day^{-1}$) was significantly highest than anytime during the incubation period. Rates during the period 4–110 days after FOM application, ranging from 3.87 to 8.75 mg $kg^{-1}day^{-1}$, were all statistically the same. In the chicken manure-treated soils, rates were significant from each other in the 0–3-, 4–13, and 14–21-day periods. Starting from 22 days after incubation, CO_2 evolution rates did not vary significantly. These results were in agreement with earlier reports. Addition of manure increased CO_2 flux of the soils and that the largest difference between manured and control soils occurred at week 1, when the manured soils had from 42 to more than 400 % higher CO_2 fluxes (Calderon et al., 2004). Similarly, after a short lag phase (3 days) after cellulose addition, the cellulose decomposition followed an exponential dynamic until the rate of CO_2 production had markedly decreased (at day 17) likely due to cellulose exhaustion (Fontaine *et al.*, 2004). Conversely, cumulative values of evolved CO_2-C increased rapidly from day 0 to 14, thereafter the increase was less for the rest of the incubation (Rudrappa *et al.*, 2006). Maximum CO_2 production rate in the urine+dairy farm effluent-applied soils incubated at 28° C was attained starting immediately after application until day 5 (Clough and Kelliher, 2005).

Table 3. Effects of time and fresh organic matter application on the CO_2 evolution rate in the 0–5- and 5–20-cm layers of Tsumagoi soil, Gunma Prefecture, Japan.

	CO_2 evolution rate (mg $kg^{-1}day^{-1}$)					
	0–5-cm			5–20-cm		
Days after FOM addition	No OM (control)	Leaf litter	Chicken manure	No OM (control)	Leaf litter	Chicken manure
0–3	22.77 a	25.89 a	68.24 a	19.26 a	23.03 a	121.67 a
4–13	5.34 b	7.8 b	74.55 a	6.29 ab	8.2 b	107.25 b
14–21	5.24 b	7.55 b	36.3 b	7.52 ab	8.75 b	49.31 c
22–44	2.33 b	4.45 b	8.27 c	5.43 ab	5.33 b	17.94 d
45–70	2.28 b	3.27 b	7.45 c	3.27 b	4.56 b	10.79 d
71–85	3.35 b	3.76 b	7.89 c	2.09 b	5.6 b	12.28 d
86–110	1.54 b	2.38 b	4.49 c	3.56 b	3.87 b	8.4 d

In a column, means followed by different letters are significant at 5% level using DMRT

Rates of organic matter decomposition depend upon several factors, ranging from the type of organic amendments to the soil type and properties, the climatic conditions and land management practices (Pedra *et al.*, 2007). In addition, the quantity and nature of the soil clay affects the amount of C stabilized in soil, since fine textures soils often contain higher amounts of OM than sandy soils (Mtambanengwe *et al.*, 2004)

Sources of CO_2 efflux from soils

According to Kuzyakov (2006), there are four main contributors to CO_2 efflux classified as microbial: (1) microbial decomposition of soil organic matter in root free soil without undecomposed plant remains, frequently referred to as "basal respiration"; (2) microbial decomposition of soil organic matter in root affected or plant residue affected soil, called "rhizosphere priming effect" or "priming effect"; (3) microbial decomposition of dead plant remains; and (4) microbial decomposition of rhizodeposits from living roots, called "rhizomicrobial respiration".

Root respiration is and the dissolution of calcium carbonate ($CaCO_3$) also contributes to CO_2 efflux from soils. However, this $CaCO_3$ contribution during pedogenesis is only marginal since soil-CO_2 flux measurements are usually done in sub-annual, annual, and decadal time scales.

Soil microbial biomass carbon

The soil microbial biomass as an active soil organic matter (SOM) fraction and agent of CO_2 production in soil is divided into two main groups: heterotrophic and autotrophic organisms. The most important heterotrophs in the soil can be subdivided into two broad groups: (1) soil microorganisms (bacteria, fungi, actinomycetes and protozoans) and (2) soil macrofauna, the contribution of which to total CO_2 efflux from soils is usually a few percent (Ke *et al.*, 2005; Konate *et al.*, 2003; Andren and Schnurner, 1985). Most of the CO_2 evolved by heterotrophic soil organisms is respired by microorganisms such as bacteria, non-mycorrhizal and mycorrhizal fungi, and actinomycetes. This component of soil CO_2 flux is collectively called microbial respiration (Kuzyakov, 2006).

The SMBC increased dramatically in the early stages of incubation (Figure 3). The application of chicken manure caused a greater increase in the SMBC than did the application of leaf litter. Peak microbial growth occurred 13 days after the application of FOM. SMBC concentration in the 5–20-cm layer that received chicken manure peaked at 1509.2 mg kg^{-1}. The 0–5-cm layer that received chicken manure peaked at a SMBC concentration of 1059.5 mg kg^{-1}. In soils that were treated with leaf litter, the peak SMBC concentration was 631.2 and 886.9 mg kg^{-1} for the 0–5- and 5–20-cm layers, respectively. Control soils had SMBC peaks of 123.62 (0–5-) and 875.16 mg kg^{-1} (5–20-) also at 13 days after FOM application.

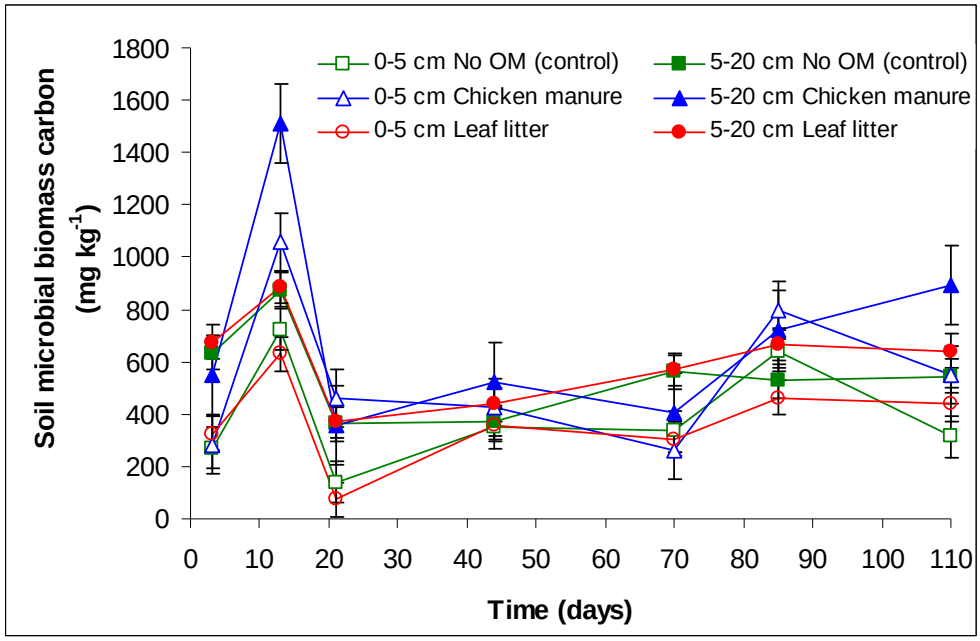

Figure 3. Changes in the soil microbial biomass (SMBC) (mg kg^{-1}) of Tsumagoi soil, Gunma Prefecture, Japan over 110 days of incubation following application of leaf litter and chicken manure.

The peak in SMBC at 13 days after the addition of FOM was followed by a drop at day 21. From day 21, different patterns in SMBC were observed. SMBC in the 0–5-cm layer of control and leaf litter-applied soils generally increased again at 44 days after incubation and peaked 85 days after FOM application while in the chicken manure-applied soil, SMBC continued to drop until day 70 and peaked at day 85. In 5–20-cm layer control, SMBC peaked at day 70, while for the leaf litter- and chicken manure-applied soils, SMBC continued to increase until the end of incubation.

In all FOM treatments, the increase in SMBC during the 4–13-day period was highest (Table 4). Following this peak was a decline at the start of the 4^{th} week (22 days after FOM addition). Following this decline was a significant increase again. For the control and leaf litter-applied soils, this was observed starting from 45 days after incubation onwards. In the case of chicken manure-applied soils, the marked increase of SMBC for the second time was observed starting from 71 days until the end of incubation.

Table 4. Effect of time and fresh organic matter application on the soil microbial biomass carbon (SMBC) of Tsumagoi soil, Gunma Prefecture, Japan.

Days after FOM addition	SMBC (mg kg^{-1})		
	No OM (control)	Leaf litter (1.81 g kg^{-1})	Chicken manure (2.12 g kg^{-1})
0–3	451.84 b	500.98 b	416.46 c
4–13	799.39 a	759.09 a	1284.36 a
14–21	249.96 d	223.12 d	409.2 c
22–44	357.9 cd	397.58 c	474.45 c
45–70	448.23 bc	436.22 bc	331.41 c
71–85	583.57 b	565.11 b	761.82 b
86–110	483.47 b	544.28 b	817.52 b

In a column, means followed by different letters are significant at 5% level using DMRT

According to Fontaine *et al.,* (2004) the supply of cellulose highly stimulated the microbial activity. In their experiment, the production of unlabelled extra CO_2 induced by glucose was completed after 3 days and amounted to about 15-19 % of the microbial biomass-C. Further, the addition of cellulose as small as <5 % of the native soil C induced a two-fold increase of total biomass, which was not sustainable, since it decreased starting from day 21 until the end of incubation. The soils amended with chicken manure showed exceptionally high CO_2 production, indicating the presence of readily available C for microbial consumption and cell division. This is revealed by the lower C/N ratio of the chicken manure compared to that of the leaf litter.

The occurrence of two distinct peaks in SMBC was seen in all treatments. The first SMBC peaks occurred 13 days after FOM application. These peaks coincided with the period of high CO_2 evolutions. Several authors stated the direct relationship between CO_2 production and SMBC (Kuzyakov *et al.,* 2000). Similar results have been reported in previous studies (e.g. Calderon *et al.,* 2004).

However, the timing of occurrence of the second SMBC peaks was different. The second SMBC peaks in the 0–5-cm depth occurred at day 70 in all treatments, while in the 5–20-cm layer, SMBC in chicken manure-applied soils continued to increase from day 70 until the end of incubation; the control had second peak at day 70 and the leaf litter-applied soil had sustained SMBC increase from day 22 until day 110.

The first SMBC peak 13 days after incubation was most likely due to the availability of substrates originating from the FOMs and from the labile SOM in the case of the control soils. The drop in SMBC at day 21 was probably due to the exhaustion of these readily-available substrates.

The occurrence of a second peak suggests that soil microorganisms started to use an alternative source of energy, since the readily-available components of the applied manure could have been used up, leading to a drop in SMBC at 21 days after FOM application. In this scenario, although the actual microbial structure that caused the first SMBC peak was not identified, we propose that the dominant microbial structure that caused the first SMBC peak was different from that in the second peak.

Several authors observed similar trend in terms of the surge in SMBC early in the incubation period. Annual application of manure caused a rapid increase in SMBC following application and potentially mineralizable C reached maximum fluxes within a month after manure application (Lee *et al.*, 2007). The microbial population is easily activated even by trace amounts of readily-available source of energy. Trace amounts of simple and easily degradable substances such as glucose or amino acids, and more complex soil and root extracts, could shift the soil microorganisms from dormancy to activity, causing more to be evolved as CO_2 than was contained in the substrate (De Nobili *et al.*, 2001). This response of the microbial biomass is presumably in anticipation of the coming of a bigger source of energy available for further reproduction and respiration. This could partly explain the response of SMBC almost immediately after FOM application. In conditions without any external application of readily-available substrates, favorable conditions of soil moisture or aeration would trigger this initial microbial response.

Kinetics and dynamics of the mineral-associated organic carbon

Original MAOC level was lower in the 0–5-cm layer (33.93 g kg^{-1}) than in the 5–20-cm layer (38.19 g kg^{-1}) of the Tsumagoi soil (Table 5).

Table 5. Initial total organic carbon (TOC) and mineral-associated organic carbon (MAOC) of the 0–5- and 5–20-cm layers of Tsumagoi soil, Gunma Prefecture, Japan.

Depth (cm)	TOC (g kg^{-1})	MAOC (g kg^{-1})	Labile SOC* (g kg^{-1})
0–5	70.57	33.93	36.64
5–20	88.9	38.19	50.71

** TOC less MAOC*

The short-term kinetics of MAOC of Tsumagoi soil are shown in Figure 4. The behavior of the MAOC three days after the application of FOM is significant (Table 6) and an interesting point of discussion. MAOC is conventional understood as a stable entity and have long turnover times due to protection by silt and clay. Statistical comparison of treatments means challenge our conventional knowledge of the stability of MAOC. There was strong evidence that significant portion of MAOC is turned over in short time scale. The decline in MAOC three days after FOM addition suggests that a portion of MAOC is prone to turnover in a matter of days, though it is believed that MAOC has turnover times of centuries to millennial timescales (Table 1).

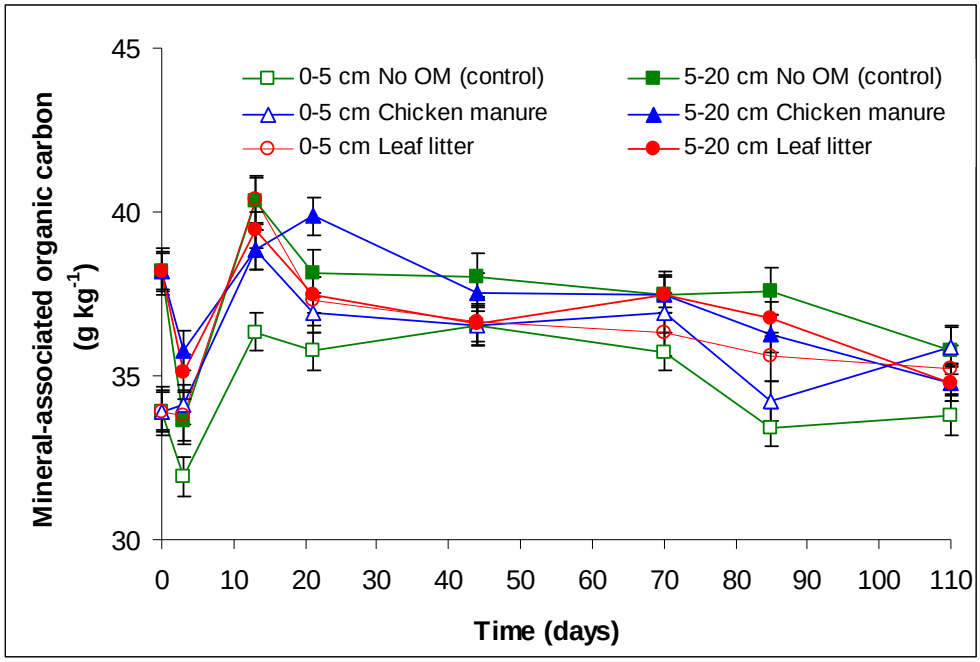

Figure 4. Mineral-associated organic carbon (MAOC) (g kg^{-1}) of Tsumagoi soil, Gunma Prefecture, Japan over 110 days following application of leaf litter and chicken manure.

Results showed that the mineral-associated organic carbon changes from time to time within a short time scale, indicating that there are sites within the mineral phase that are accessible to the microorganisms. It may be safe to say that the mineral phase is also continually interacting with the soil organic matter, as indicated by the significant differences in MAOC at particular time periods.

Between measurement dates during the incubation period, “add and subtract” changes in the MAOC particularly in the early stage of incubation were observed. These changes could have been due to the labile SOM that moves and associates with the particle size fractions. SOM is a continuum of materials from very young to very old with ongoing transfers between pools (Haile-Mariam *et al.*, 2008). This means that SOM moves between particle size fractions. Owing to artificial, biological, and other pedoturbations, the transfer of SOC between the particle size fractions is a continuous process in the soil continuum. However, it is assumed that the transfer of SOC from the silt- and clay sized fractions should be less than the transfer from the sand fractions to the finer-sized fractions, due to the physical protection of SOM by the silt and clay fractions (Hassink, 1997; can Veen and Kuikman, 1990). The organo-silt and organo-clay fractions in FOM are slow to mineralize due to physical protection (Mando *et al.*, 2005). This could result to the heterogeneity of SOC in the fine soil fractions because SOC from the sand-size fraction, from where SOM moves to the silt- and clay-sized fractions, is dominated by particulate plant material that has a lower extent of decomposition (Guggenberger *et al.*, 1995) and has younger radiocarbon ages (Lützow *et al.*, 2006).

Most notable was the significant decline of the MAOC three days after FOM addition compared with the day zero level (Table 6). Two possible fates of the lost MAOC can be interpreted – (1) some microbial structures utilized this MAOC for microbial cell division, and (2) microbial degradation to CO_2. This cannot be verified using the experimental design used in this experiment because to prove this may require isotopic fractionation of evolved CO_2 and comparing the isotopic signature of the MAOC. This finding, however, proved that the stable MAOC may be a source of microbial energy in the short-term, although this stable fraction is conventionally believed to have long mean residence and turnover times.

Table 6. Effect of time on the mineral-associated organic carbon (MAOC) of Tsumagoi soil, Gunma Prefecture, Japan.

Days after FOM addition	MAOC ($g\ kg^{-1}$)
0	36.06 cd
3	34.05 e
13	39.03 a
21	37.58 b
44	36.99 bc
70	36.91 bc
85	35.64 d
110	35.64 d

Means followed by different letter(s) are significant at 5% level by DMRT

Conclusions

The occurrence of second SMBC peaks in this experiment involving one-time only addition of fresh organic matters is very meaningful, and suggests a shift in the microbial community structure as the readily-available substrates from FOM became exhausted a few days after application. This suggests that the new soil microbial biomass growth found energy from a new source, which could be the MAOC, a stable SOM fraction. Regarding this process, it is suggested that most energetic compounds of FOM are used by r-strategist microorganisms that only decompose FOM. K-strategists arise only in the later stage of the FOM decomposition process when energy-rich compounds have been exhausted and only polymerized compounds remain (Fontaine *et al.*, 2003).

Our finding of a significant MAOC decline three days after FOM application puts into question the convention that only the labile SOC contributes to CO_2 evolution in soils applied with FOM. Further, this suggests that physical protection of SOC in the silt and clay fractions is not a guarantee of its resistance to turnover in the short-term time scale, although previously believed

as such. This could have big impact on the overall terrestrial carbon dynamics if the most stable SOC with long turnover times are lost in exchange of the less stable SOC that moves into the fine soil fractions during carbon input to soil.

References

Andren, O., and J. Schnurer, 1985. Barley Straw Decomposition with Varied Levels of Microbial Grazing by *Folsomia fimetaria*(L.) (*Collembola isotomidae*). Oecologia, 68: 57–62.

Baldock J. A., and J. O. Skjemstad, 2000. Role of the Soil Matrix and Minerals in Protecting Natural Organic Materials Against Biological Attack. Organic Geochemistry, 31:697–710.

Bell, J. M., J. L. Smith, V. L. Bailey, and H. Bolton, 2003. Priming Effect and C Storage in Semi-arid No-till Spring Crop Rotations. Biology and Fertility of Soils, 37:237–244.

Calderon F. J., G. W. McCarty, J. A. S. van Kessel, J. B. Reeves, 2004. Carbon and Nitrogen Dynamics during Incubation of Manured Soil. Soil Science Society of America Journal, 68:1592–1599.

Cambardella, C. A., and E. T. Elliot, 1992. Particulate Soil Organic Matter Changes Across a Grassland Cultivation Sequence. Soil Science Society of America Journal, 58:1167–1173.

Campbell, C. A., G. P. Lafond, R. P. Zentner, and V. O. Biederbeck, 1991. Influence of Fertilizer and Straw Baling on Soil Organic Matter in a Thick Black Chernozem in Western Canada. Soil Biology and Biochemistry, 23:443–446.

Clough T. J., and F. M. Kelliher, 2005. Dairy Farm Effluent Effects on Urine Patch Nitrous Oxide and Carbon Dioxide Emissions. Journal of Environmental Quality, 34:979–986.

Dalenberg, J. W., and G. Jager, 1989. Priming Effect of some Organic Additions to ^{14}C-labelled Soil. Soil Biology and Biochemistry, 21:443–448.

De Nobili, M., M. Contin, C. Mondini, and P. C. Brookes, 2001. Soil Microbial Biomass is Triggered into Activity by Trace Amounts of Substrate. Soil Biology and Biochemistry, 33:1163–1170.

Dumale, W. A. Jr., T. Miyazaki, T. Nishimura and K. Seki, 2009. CO_2 Evolution and Short-term Carbon Turnover in Stable Soil Organic Carbon from Soils Applied with Fresh Organic Matter. Geophysical Research Letters. 36:L01301.

Foereid, B., A. de Neergaard, and H. H. Jensen, 2004. Turnover of Organic Matter in a *Miscanthus* field: Effect of Time in *Miscanthus* Cultivation and Inorganic Supply. Soil Biology and Biochemistry, 36:1075–1085.

Fontaine S., G. Badoux, L. Abbadie, and A. Mariotti, 2004. Carbon Input to Soil may Decrease Soil Carbon Content. Ecological Letters, 7:314–320.

Fontaine S., A. Mariotti, and L. Abbadie, 2003. The Priming Effect of Organic Matter: A Question of Microbial Competition? Soil Biology and Biochemistry, 35:837–843.

Gerzabek, M. H., G. Haberhauer, and H. Kirchman, 2001. Soil Organic Matter Pools and Carbon-13 Natural Abundances in Particle Size Fractions of a Long-term Agricultural Field Experiment Receiving Organic Amendments. Soil Science Society of America Journal, 65:352–358.

Gerzabek, M. H., F. Pichlmayer, H. Kirchmann, and G. Haberhauer, 1997. The Response of Soil Organic Matter to Manure Amendments in a Long-term Experiment at Ultuna, Sweden. European Journal of Soil Science, 48:273–282.

Guggenberger, G., W. Zech, L. Haumaier, B. T. Christensen, 1995. Land-use Effects on the Composition of Organic Matter in Particle-size Separates of Soils: II. CPMAS and Solution ^{13}C NMR Analysis. European Journal of Soil Science, 46:147–158.

Haile–Mariam, S., H. P. Collins, S. Wright, and E. A. Paul, 2008. Fractionation and Long–term Laboratory Incubation to Measure Soil Organic Matter Dynamics. Soil Science Society of America Journal, 72:370–378.

Hamer, U., and B. Marschner, 2005. Priming Effects in Soils after Combined and Repeated Substrate Additions. Geoderma, 128:38–51.

Hassink, J., 1997. The Capacity of Soils to Preserve Organic C and N by their Association with Clay and Silt Particles. Plant and Soil, 191:77–87.

Ke, X., K. Winter, and J. Filser, 2005. Effects of Soil Mesofauna and Farming Management on Decomposition of Clover Litter: A Microcosm Experiment. Soil Biology and Biochemistry, 37:731–738.

Konate, S., X. Le Roux, B. Verdier, and M. Lepage, 2003. Effect of underground Fungus-growing Termites on Carbon Dioxide Emission at the Point- and Landscape-scales in an African Savanna. Functional Ecology, 17: 305–314.

Kuzyakov, Y., 2006. Sources of CO_2 Efflux from Soil and Review of Partitioning Methods. Soil Biology and Biochemistry, 38:425–448.

Kuzyakov, Y., J. K. Friedel, and K. Stahr, 2000. Review of Mechanisms and Quantification of Priming Effects. Soil Biology and Biochemistry, 32:1485–1498.

Lee, D. K., J. J. Doolittle, and V. N. Owens, 2007. Soil Carbon Dioxide Fluxes in Established Switchgrass Land Managed for Biomass Production. Soil Biology and Biochemistry, 39:178–186.

Li, L. X. Zhang, P. Zhang, J. Zheng and G. Pan, 2007. Variation of Organic Carbon and Nitrogen in Aggregate Size Fractions of a Paddy Soil under Fertilization Practices from Tai Lake Region, China. Journal of Food Science and Agriculture, 87:1052–58.

Lützow, M. V., I. Kőgel–Knabner, K. Ekschmitt, E. Matzner, G. Guggenberger, B. Marschner and H. Flessa, 2006. Stabilization of Organic Matter in Temperate Soils: Mechanisms and their Relevance under Different Soil Conditions – A Review. European Journal of Soil Science, 57:426–45.

Mando, A., B. Quattara, A. E. Somado, M. C. S. Woperis, L. Stroosnijder, H. Breman, 2005. Long-term Effects of Fallow, Tillage and Manure Application on Soil Organic Matter and Nitrogen Fractions on Sorghum Yield under Sudano-Sahelian Conditions. Soil Use and Management, 21:25–31.

Mtambanengwe, F., P. Mapfuno, and H. Kirchmann, 2004. Decomposition of organic matter in soil as influenced by texture and pore size distribution. In Managing nutrient cycles to sustain soil fertility in sub-Saharan Africa Ed., Bationo, A. Centro Internacional de Agriculture Tropical. pp: 261–276.

Ohm, H., U. Hamer, and B. Marschner, 2007. Priming Effects in Soil Size Fractions of a Podzol Bs Horizon after Addition of Fructose and Alanine. Journal of Plant Nutrition and Soil Science, 170:551–59.

Parton W. J., J. W. B. Stewart, and C. V. Cole, 1988. Dynamics of C, N, P, and S in Grassland Soils: A Model. Biogeochemistry, 5:109–131.

Pedra, F., A. Polo, A. Ribiero, and H. Domingues, 2007. Effects of Municipal Solid Waste Compost and Sewage Sludge on Mineralization of Soil Organic Matter. Soil Biology and Biochemistry, 39: 1375–1382.

Rudrappa L., T. J. Purakayastha, D. Singh, and S. Bhadraray, 2006. Long-term Manuring and Fertilization Effects on Soil Organic Carbon Pools in a Typic Haplustert of Aemi-arid Sub-tropical India. Soil Tillage and Research, 88:180–192.

Rumpel, C., H. Knicker, I. Kőgel-Knabner, J. O. Skjemstad and R. F. Huttl, 1998. Types and Chemical Composition of Organic Matter in Reforested Lignite-rich Mine Soils. Geoderma, 86:123–142.

Sherrod L. A., G. A. Peterson, D. G. Westfall, and L. R. Ahuja, 2005. Soil Organic Carbon after 12 Years in No-till Dryland Agroecosystems. Soil Science Society of America Journal, 69:1600–1608.

van Veen, J. A., and P. J. Kuikman, 1990. Soil Structural Aspects of Decomposition of Organic Matter by Microorganisms. Biogeochemistry, 11:213–233.

Vance, E. D., P. C. Brookes, and D. S. Jenkinson, 1987. An Extraction Method for Measuring Soil Microbial Biomass C. Soil Biology and Biochemistry, 19:703–707.

Zhao, L., Y. Sun, X. Zhang, X. Yang, and C. F. Drury, 2006. Soil Organic Carbon in Clay and Silt Sized Particles in Chinese Mollisols: Relationship to the Predicted Capacity. Geoderma, 132:315–323.