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Applied Soil Ecology 33 (2006) 3–9
Applied Soil Ecology
www.elsevier.com/locate/apsoil

Changes of natural ^{13}C abundance in microbial biomass during litter decomposition

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Received 12 November 2004; received in revised form 23 September 2005; accepted 23 September 2005

Abstract

The carbon (C) isotopic composition of soil microbial biomass (SMB) was measured in litter-amended and control plots located at three different elevations (280–2210 m, sea level) during the decomposition of plant litter (*Vicia villosa*) added to upland soils in southwest China. Fourteen months around the addition of litter, soil microbial biomass carbon (SMBC) was followed during experiments. Results showed that SMBC was significantly higher in the litter-amended plots than the controls at the two lowest but not the highest elevation and there was no corresponding difference in microbial $\delta^{13}C$ values at the same time. However, microbial $\delta^{13}C$ values at the two lowest sites were significantly higher in the litter-amended plots immediately following the peak of microbial C. The $\delta^{13}C$ value of SMBC in litter-amended plot was higher than that in control plot, indicating that the degree of microbial decomposition and quality of plant litter will effect on shift of $\delta^{13}C$ values of SMBC, which may be mainly caused by microbial selective utilization of organic compounds. The sequence of magnitude of $\delta^{13}C$ value of SMBC was consistent with that of soil organic carbon (SOC) among three experiment sites, indicating that the $\delta^{13}C$ value of SMBC reflects gross changes in the $\delta^{13}C$ value of SOC in the corresponding samples.

Keywords: Soil microbial biomass carbon; $\delta^{13}C$ values; Soil organic matter; Decomposition of plant litter

1. Introduction

Most soil organic matter is derived from plant litter, hence the $^{13}C/^{12}C$ ratio of the litter has a direct impact on the ^{13}C content of soil organic matter (Van Kessel et al., 1994). All plant litter carbon (C) passes through the soil microbial biomass (SMB), at least once, as it is transferred from fresh litter to soil organic matter. The litter decomposition rates were controlled by climate, litter quality and soil organism (Smith and Bradford, 2003). Although soil microbial biomass comprises <5% of soil organic matter, it performs critical functions in soil and environment (Dalal, 1998). ^{13}C measurements and fumigation–extraction methods can be used to investigate soil microbial biomass turnover (Ryan and Aravena, 1994), but the shift in isotopic composition of soil organic matter during aerobic microbial degradation (−3.7 to +1.4 parts per thousand, ‰) (Šantrůčková et al., 2000) could seriously confound interpretations.

There are differing opinions regarding the origin of the shift in the isotopic composition of organic matter during microbial decomposition, a large fractionation against ^{13}C during microbial decomposition of added organic material (Blair et al., 1985), and during microbial-mediated respiration and decomposition of soil organic matter can enrich soils with ^{13}C (Smejkal

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doi:10.1016/j.apsoil.2005.09.006

et al., 1971; Stout et al., 1981; Macko and Estep, 1984). Since decomposition is a microbially mediated process, a discrimination against ^{13}C during plant litter decomposition could result in an increase in the $\delta^{13}C$ value of the soil with depth (Conteh et al., 1997). Other studies have concluded, however, that discrimination against heavier C isotopes by microbes during decomposition is negligible, amounting to 0.5‰ or less (Campbell et al., 1967; Monson and Hayes, 1982), and that no strong ^{13}C discrimination during microbial respiration occurred when simple substrates were decomposed (Ekblad et al., 2002). The shifts in isotopic signature at advanced stages of litter decomposition are not due to differential loss of constituent C fractions or to fractionations during microbial transformation, but rather to mixing of external C with original litter C, presumably via fungal hyphae or microbial populations (Wedin et al., 1995). The shift of C isotopic composition could also be the result of the temperature-dependent shift in the active microbial community causing a shift in the C pool being mineralized (Andrews et al., 2000).

The decomposition of organic matter is a key process in soil ecosystem functioning, for which stoichiometric relations between plant litter and consumers play important role (Hessen et al., 2004). In this paper, we present a field experiment in which plant litter contained relatively higher nitrogen (N) concentration was added to upland soils located at three different elevations. We tested the hypothesis that changes of soil microbial C concentrations and $\delta^{13}C$ values in microbial biomass responded differently to time since addition of plant litter to the soils because of different litter quality and temperature in both litter-amended and control plots at each site.

2. Materials and methods

2.1. The study site

Three terrace fields on slopes at different elevations above sea level were chosen as experimental sites (Table 1). The sites were in mountainous terrain and in a transition zone from lowland to hills in the eastern part of the Yunnan–Guizhou Plateau, southwest China. The field plots were approximately 200 m^2 in area and were divided in two sections, one as a litter-amended, the other as an unamended reference plot at each elevation site. Experimental plant litter was *Vicia villosa*, a legume. Generally, *V. villosa* was planted at autumn and was incorporated into soil as a fertilizer at spring of the next year in southwest China. In this experiment plant litter was added on 6 April at Xiaohai, 9 April at Jinzhu and 12 April 2001 at Bakai. Chopped litter of *V. villosa* was added in each experimental plot at 5-cm depth (5 kg litter m^{-2}). The plant seed came from the highest elevation site, but grown in areas adjacent each experimental plot at autumn ahead because plant $\delta^{13}C$ values increased with elevation. All plots were regularly cleared of weeds and black fallow after addition of litter. The $\delta^{13}C$ values and N concentrations of amended litter, weeds, and some of the vegetable and crop plants rotated before experiment are given in Table 2. We sampled the soil 11 times at Xiaohai, 14 times at Jinzhu and 13 times at Bakai during the study, which lasted from 5 January 2001 to 25 February 2002. At each sampling time, three 5-cm diameter cores were taken randomly, extending from the surface to 10-cm depth from each litter-amended and control plot. Visible plant fragments and stones (>2 mm) were removed and the soil was mixed for determining microbial biomass C, and then air-dried for determining soil organic C, etc.

2.2. Laboratory analysis

We acidified the air-dried soil samples to remove any residual $CaCO_3$ before analysis of soil organic C isotopic composition (Bashkin and Binkley, 1998). The soil samples were then oven-dried at 60 °C to constant mass and ground. Total N and organic C were determined with a CHNS autoanalyser (PE 2400-ll).

Microbial biomass C was determined on field-moist samples in duplicate by the chloroform fumigation–extraction technique (Voroney et al., 1993). Briefly, 25 g

Table 1
Description of the three experimental sites and soils in Guizhou province of southwest China

Location	Position	Sea level elevation (m)	Annual average air temperature (°C)	Annual precipitation (mm)	Organic C (g kg^{-1})	$\delta^{13}C$ of soil organic C (‰)	Total N (g kg^{-1})	Clay (%)
Xiaohai	26°56′N, 104°10′E	2210	10.4	1028	21.5	−23.9	2.4	28
Jinzhu	26°30′N, 106°39′E	1140	14.3	1122	17.0	−22.5	1.7	61
Bakai	25°49′N, 108°30′E	280	18.1	1200	13.5	−24.0	2.3	42

Table 2
The average $\delta^{13}C$ values and N concentration of plant materials

Plants	$\delta^{13}C$ (‰)	N (%)
Xiaohai		
Green manure	−27.5	4.1
Maize	−11.4	
Weeds	−26.3	2.9
Potato (stem)	−27.0	0.8
Jinzhu		
Green manure	−27.8	3.8
Maize	−11.4	
Weeds	−28.9	2.0
Sweet potato	−27.4	1.0
Bakai		
Green manure	−30.4	4.4
Maize	−11.4	
Rape	−30.4	1.0
Chinese cabbage	−30.8	3.8
Radish	−30.6	3.1
Sweet potato	−28.1	0.8

soil was fumigated 24 h at 25 °C and subsequently extracted with 100 ml 0.5 M K_2SO_4 for 1 h. The unfumigated control soil was extracted in the same manner. Organic C in the extracts was measured by dichromate oxidation and microbial biomass C was calculated according to Voroney et al. (1993). The remaining parallel extracts were combined and dried at 60 °C. The $\delta^{13}C$ values of dry extracts, some of which were compared to freeze-dried extracts and plant samples were measured by combustion of approximately 2 mg C mixed with CuO (1:50) at 550 °C in the vacuum–combustion system for 12 h (at 850 °C 2 h for soil samples). Carbon dioxide (CO_2) generated in the combustion tubes was separated by cryogenic distillation, collected in breakseals and analyzed on a mass spectrometer (MAT 252 Finnigan, Bremen, Germany). The data are expressed relative to the international standard PDB (as $\delta^{13}C$‰).

The $\delta^{13}C$ of the microbial biomass was estimated as the $\delta^{13}C$ in the extract from the fumigated sample in excess of that extracted from the control sample. That is (Ryan and Aravena, 1994)

$$\delta^{13}C = \frac{\delta^{13}C_{FUM} \times C_{FUM} - \delta^{13}C_{CONT} \times C_{CONT}}{C_{FUM} - C_{CONT}} \quad (1)$$

where C_{FUM} and C_{CONT} were the amounts of C extracted from the fumigated and unfumigated control samples, respectively, and $\delta^{13}C_{FUM}$ and $\delta^{13}C_{CONT}$ represented the ^{13}C composition of the fumigated and control extracts, respectively.

2.3. Statistical analysis

Twenty percentage of all extracts measured in duplicate for C isotope composition, others in single, and the standard deviation of the duplicates did not exceed 0.2‰. Differences between mean values of $\delta^{13}C$ and microbial C concentrations for locations, treatments and time were tested for their significance by using linear regression and variance analysis at $P = 0.05$.

3. Results

3.1. Response of soil microbial biomass C (SMBC) after addition of plant litter

An increase in microbial biomass C concentration after the addition of litter was faster at Bakai and Jinzhu sites than at the high-elevation Xiaohai site (Fig. 1). At Bakai the largest difference in microbial C between the litter-amended and control plots occurred on 28 May 2001, 47 days after the addition of litter. At Jinzhu the

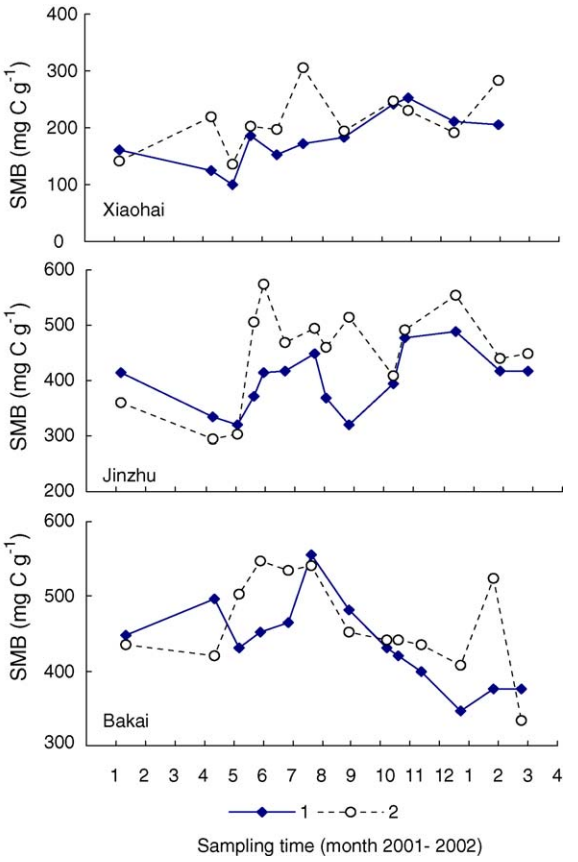

Fig. 1. The changes of carbon concentrations in soil microbial biomass (SMB) in litter-amended and control plots during experiments (1) control and (2) litter-amended.

Table 3
Average carbon concentration and $\delta^{13}C$ values of soil microbial biomass (mean ± S.D.) over whole experimental period of 14 months

	SMB (μg C g^{-1})			$\delta^{13}C$ (‰)		
Site	Xiaohai	Jinzhu	Bakai	Xiaohai	Jinzhu	Bakai
Sampling number	11	14	13	11	14	13
Control site	180.9 ± 46.3	400.2 ± 52.7	436.8 ± 56.5	−23.1 ± 0.5	−22.8 ± 0.5	−24.0 ± 0.4
Amended site	213.7 ± 52.1	451.3 ± 85.1	462.8 ± 62.9	−23.0 ± 0.5	−22.5 ± 0.5	−23.9 ± 0.4
Paired *t*-test	NS	NS	NS	NS	NS	NS

NS, not significant.

peak in SMB carbon occurred on 30 May 2001, 51 days after the addition of litter, but at Xiaohai the peak did not occur until 12 June 2001, 96 days after the addition (Fig. 1). The average concentration of soil microbial biomass C during the cause of the experiment was highest at Bakai, followed by Jinzhu and lowest at Xiaohai in both the litter-amended and control plots (Table 3). The mean concentrations of SMBC in the litter-amended and control plots at each site did not differ significantly only the whole experimental period (Table 3). However, microbial C concentrations differed between the litter-amended and control plots at the Bakai and Jinzhu sites at the three consecutive sampling dates following amendment with the highest concentration of SMB carbon (Table 4). Differences between the three subsequent sampling dates were not significant (Table 5).

3.2. Carbon isotope composition of microbial biomass after addition of plant litter

The average $\delta^{13}C$ value of the microbial biomass calculated from all sampling periods did not differ significantly between the litter-amended and control plots at any of the sites (Table 3) "NS". Their average values followed the order: Jinzhu > Xiaohai > Bakai (Table 3), which is similar to the order of $\delta^{13}C$ values in the whole soils (Table 1). Concentrations of carbon in SMB following the addition of litter increased at the Jinzhu and Bakai sites but there was no corresponding increase in microbial $\delta^{13}C$ values at the same time (Fig. 2, Table 4). However, in the subsequent months significant differences in microbial $\delta^{13}C$ values between the litter-amended and control plots occurred at Jinzhu and Bakai despite that the differences in SMBC found previously were not present any longer (Fig. 2, Table 5).

4. Discussion

It was apparent that the plant $\delta^{13}C$ values increased with elevation (Table 2). Plant litter using this study was legume, which could cause the litter-amended plot to differ from the control plot for the quantity and nitrogen concentration of litter incorporated into soil at each site. The microbial biomass C concentrations were higher when plant litter was incorporated into the experiment plot (Fig. 1, Table 4). The increases of microbial biomass C with decreasing elevation at our sites (Fig. 1, Table 3) could be ascribed to greater inputs of plant litter, and to higher air temperature, with decreasing elevation, which is similar to our previous results (Piao et al., 2000, 2001).

The average microbial $\delta^{13}C$ values over the experimental period that showed the same site differences (Jinzhu > Xiaohai > Bakai) as those of the whole soil $\delta^{13}C$ values (Tables 1 and 3) although not significant are in accordance with Šantrůčková et al.

Table 4
Carbon concentrations and $\delta^{13}C$ values of soil microbial biomass at the three sampling dates with the highest concentration of SMBC after addition of plant litter (mean ± S.D.)

	SMB (μg C g^{-1})			$\delta^{13}C$ (‰)		
	Xiaohai[a]	Jinzhu[b]	Bakai[c]	Xiaohai[a]	Jinzhu[b]	Bakai[c]
Number	3	3	3	3	3	3
Control site	170.1 ± 13.5	401.8 ± 20.6	448.4 ± 14.0	−22.7 ± 0.3	−22.6 ± 0.2	−24.4 ± 0.4
Amended site	233.1 ± 51.5	516.5 ± 43.1	528.3 ± 18.6	−22.9 ± 0.3	−22.5 ± 0.2	−24.3 ± 0.3
Paired *t*-test	NS	$P < 0.05$	$P < 0.01$	NS	NS	NS

[a] Sampling time: 6.15, 7.12, 8.24 (month day, 2001).
[b] Sampling time: 5.19, 5.30, 6.20 (month day, 2001).
[c] Sampling time: 5.5, 5.28, 6.25 (month day, 2001).

Table 5
Concentrations and $\delta^{13}C$ values of soil microbial biomass carbon (SMBC) calculated at the three sampling dates following the litter-induced increase in SMBC (mean ± S.D.)

	SMB ($\mu g\ C\ g^{-1}$)			$\delta^{13}C$ (‰)		
Site	Xiaohai[a]	Jinzhu[b]	Bakai[c]	Xiaohai[a]	Jinzhu[b]	Bakai[c]
Sampling number	3	3	3	3	3	3
Control site	234.9 ± 17.2	378.7 ± 52.6	489.8 ± 51.6	−23.0 ± 0.6	−23.0 ± 0.1	−24.2 ± 0.2
Amended site	222.6 ± 23.5	489.2 ± 22.0	477.9 ± 44.7	−22.5 ± 0.2	−22.3 ± 0.2	−23.7 ± 0.1
Paired *t*-test	NS	NS	NS	NS	$P < 0.05$	$P < 0.05$

[a] Sampling time: 10.12, 10.28, 12.12 (month day, 2001).
[b] Sampling time: 7.21, 8.2, 8.26 (month day, 2001).
[c] Sampling time: 7.19, 8.29, 10.16 (month day, 2001).

(2000) that found a significant relationship between $\delta^{13}C$ of microbial biomass and that of soil organic C ($r = 0.959$, $n = 21$). However, Bird et al. (2002) indicated that there is no relation between the $\delta^{13}C$ value of soil organic carbon (SOC) and the $\delta^{13}C$ value of microbial biomass. Although the $\delta^{13}C$ values of plant litter and weeds increased with increasing elevation (Table 2), the sequence of microbial $\delta^{13}C$ values differed from that of microbial C concentrations (Table 3). This may reflect greater past inputs of C_4-crop residues, which were grown before this experiment began, because the productivity of maize was higher at Jinzhu than at both Xiaohai and Bakai sites (Piao et al., 2001).

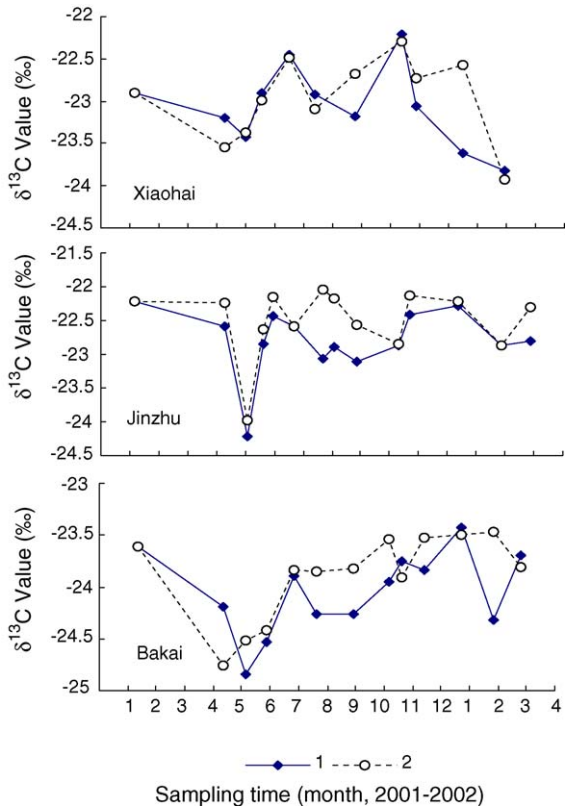

Fig. 2. The changes of $\delta^{13}C$ values of soil microbial biomass (SMB) in litter-amended and control plots during experiments (1) control and (2) litter-amended.

The $\delta^{13}C$ values of plant litter should not remain constant during decomposition if the chemical fractions of plant tissue differ in $\delta^{13}C$ values and decay at different rates (Berg et al., 1993). Any synthetic or degradative process has the potential for leaving an isotopic fingerprint on the newly created compounds (Blair et al., 1985). By mass balance considerations, if one product becomes depleted in the heavier C isotope, the other product must be enriched in that isotope (Blair et al., 1985). Therefore, a shift of the C isotope composition of plant litter towards increasing $\delta^{13}C$ values during microbial decomposition would be caused by ^{13}C discrimination or by selective utilization of organic compounds with a light C isotope. When averaged over the whole experimental period, $\delta^{13}C$ values of SMBC in the litter-amended plots relative to those of the original plant litter, were 4.4‰ greater at Xiaohai, 5.0‰ at Jinzhu and 6.4‰ at the Bakai site, calculated from Tables 2 and 5. If this $\delta^{13}C$ enrichment was caused by only discrimination or selective utilization, the $\delta^{13}C$ values of SMBC in both litter-amended and control plots at each experimental site should be equal. The experimental results showed, therefore, that the shift of carbon isotope composition of microbial biomass towards increasing $\delta^{13}C$ values were mainly caused by microbial selective utilization. The microbial population uses compounds preferentially, such as cellulose, starch and protein that have larger $\delta^{13}C$ values than the average of organic carbon in the soil (Bird et al., 2002).

Plant litter quality controls decomposition and mineralization by direct effects on the microbes (Bending et al., 1998). Carbon availability determines the growth of soil microbial biomass in arable soil, and

this biomass controls, in turn, the immobilization and release of mineral nitrogen (Van Veen et al., 1984). Because large amounts of labile C are required to drive N immobilization (Aber, 1992), immobilization should have been greatest at the beginning of our decomposition experiment. Recent results from an incubation experiment where ryegrass litter were applied also suggest that N immobilization is more sensitive than N mineralization to low temperatures (3–15 °C) (Andersen and Jensen, 2001), However, neither of these factors led to significant differences in the microbial $\delta^{13}C$ values in the litter-amended and control plots at any of our sites during the early studies of the experiment (Fig. 2, Table 4). Macko and Estep (1984) found that when microorganisms were grown on glycine and NO_3^-–N, the $\delta^{13}C$ of the cell was 6–9‰ heavier than that of the substrate. In a complex medium, biomass enriched in $\delta^{13}C$ could result from the selective assimilation of isotopically enriched C in amino acids. In our experiment, the $\delta^{13}C$ values of SMBC differed significantly at the time of immediately following the peak of microbial C between the litter-amended and control plots at the Jinzhu and Bakai sites, even though the concentrations of SMBC in the litter-amended plots did not differ from those in the control plots (Table 5). Those phenomena should be explained by the feature of the decomposition system: the decomposers start with a material rich in C and hence N limited, but as the substrate is used, C becomes limiting, the decomposers have higher N:C ratios (Sterner and Elser, 2002), and can, then, release N and other nutrients and make them available for uptake by plants again, such cycles cause the consequence that soils are relatively rich in N than C relative to plants (Hessen et al., 2004). During the initial phase of the litter decomposition evolved CO_2 was significantly enriched in new C compared with the total organic C in the soil (Hagedorn et al., 2004), and the higher carbon concentrations in microbial biomass in litter-amended plots should ascribe to the addition of new litter, but the $\delta^{13}C$ values of SMBC in litter-amended plots were similar to control plots at that time (Table 4), implying that there was significant biological selective utilization by microorganisms. N concentration increased with the plant litter decomposition processing (Moro and Domingo, 2000), resulting in the shift of carbon isotope composition in microbial biomass toward increasing $\delta^{13}C$ values in litter-amended plots (Table 5). These results clearly suggest that the shift of $\delta^{13}C$ values in microbial C depended on the degree of litter decomposition and should be related to nitrogen concentration in litter.

5. Conclusions

Because the isotopic composition of the plant litter in both litter-amended and control plot was similar at our different sites, we conclude that the observed differences between the isotopic composition of the microbial biomass and soil organic matter must be due to differences that arise from the decomposition process. The shift in the $\delta^{13}C$ values in microbial biomass due to plant litter addition at all these upland sites was only short-term and the $\delta^{13}C$ values of bulk soil organic matter hence seems to be determined by the ^{13}C content of microbial biomass in the long-term.

Acknowledgements

We thank Dr. William H. Schlesinger, Nicholas School of the Environment and Earth Sciences, Duke University, and Dr. D.J. Ross, Landcare Research, Palmerston North, New Zealand, for assistance in revising the manuscript. This study was supported by the National Natural Science Foundation of China (Grant nos. 40072100 and 40372133).

References

Aber, J.D., 1992. Nitrogen cycling and nitrogen saturation in temperate forest ecosystems. Trends Ecol. Evol. 7, 220–223.

Andersen, M.K., Jensen, L.S., 2001. Low temperature effects on short-term gross N mineralisation–immobilisation turnover after incorporation of a green manure. Soil Biol. Biochem. 33, 511–521.

Andrews, J.A., Matamala, R., Westover, K.M., Schlesinger, W.H., 2000. Temperature effects on the diversity of soil heterotrophs and the $\delta^{13}C$ of soil-respired CO_2. Soil Biol. Biochem. 32, 699–706.

Bashkin, M.A., Binkley, D., 1998. Changes in soil carbon following afforestation in Hawaii. Ecology 79, 828–833.

Bending, G.D., Turner, M.K., Burns, I.G., 1998. Fate of nitrogen from crop residues as affected by biochemical quality and the microbial biomass. Soil Biol. Biochem. 30, 2055–2065.

Berg, B., McClaugherty, C., Johansson, M.-B., 1993. Litter mass-loss rates in late stages of decomposition at some climatically and nutritionally different pine sites. Long-term decomposition in a Scots pine forest. VIII. Can. J. Bot. 71, 680–692.

Bird, M., Santrùcková, H., Lloyd, J., Lawson, E., 2002. The isotopic composition of soil organic carbon on a north–south transect in western Canada. Eur. J. Soil Sci. 53, 393–403.

Blair, N., Leu, A., Muñoz, E., Olsen, J., Kwong, E., des Marais, D., 1985. Carbon isotopic fractionation in heterotrophic microbial metabolism. Appl. Environ. Microbiol. 50, 996–1001.

Campbell, C.A., Paul, E.A., Rennie, D.A., McCallum, K.J., 1967. Factors affecting the accuracy of the carbon-dating methods in soil humus studies. Soil Sci. 104, 81–85.

Conteh, A., Lefroy, R.D.B., Blair, G.J., 1997. Dynamics of organic matter in soil as determined by variations in $^{13}C/^{12}C$ isotopic ratios and fractionation by ease of oxidation. Aust. J. Soil Res. 35, 881–890.

Dalal, R.C., 1998. Soil microbial biomass—what do the numbers really mean? Aust. J. Soil Res. 38, 649–665.

Ekblad, A., Nyberg, G., Högberg, P., 2002. ^{13}C-discrimination during microbial respiration of added C_3-, C_4- and ^{13}C-labelled sugars to a C_3-forest soil. Oecologia 131, 245–249.

Hagedorn, F., Saurer, M., Blaser, P., 2004. A ^{13}C tracer study to identify the origin of dissolved organic carbon in forested mineral soils. Eur. J. Soil Sci. 55, 91–100.

Hessen, D.O., Ågren, G.I., Anderson, T.R., Elser, J.J., de Rutter, P.C., 2004. Carbon sequestration in ecosystems: the role of stoichiometry. Ecology 85, 1179–1192.

Macko, S.A., Estep, M.L.F., 1984. Microbial alteration of stable nitrogen and carbon isotopic compositions of organic matter. Org. Geochem. 6, 787–790.

Monson, K.D., Hayes, J.M., 1982. Carbon isotopic fractionation in the biosynthesis of bacterial fatty acids. Ozonolysis of unsaturated fatty acids as a means of determining the intramolecular distribution of carbon isotopes. Geochim. Cosmochim. Acta 4, 139–149.

Moro, M.J., Domingo, F., 2000. Litter decomposition in four woody species in a Mediterranean climate: weight loss, N and P dynamics. Ann. Bot. 86, 1065–1071.

Piao, H.C., Hong, Y.T., Yuan, Z.Y., 2000. Seasonal changes of microbial biomass carbon related to climatic factors in soils from karst areas of southwest China. Biol. Fertil. Soils 30, 294–297.

Piao, H.C., Liu, G.S., Wu, Y.Y., Xu, W.B., 2001. Relationships of soil microbial biomass carbon and organic carbon with environmental parameters in mountainous soils of southwest China. Biol. Fertil. Soils 33, 347–350.

Ryan, M.C., Aravena, R., 1994. Combining ^{13}C natural abundance and fumigation–extraction methods to investigate soil microbial biomass turnover. Soil Biol. Biochem. 26, 1583–1585.

Šantrůčková, H., Bird, M.I., Lloyd, J., 2000. Microbial processes and carbon-isotope fractionation in tropical and temperate grassland soils. Funct. Ecol. 14, 108–114.

Smejkal, V., Cook, F.D., Krouse, H.R., 1971. Studies of sulfur and carbon isotope fractionation with microorganisms isolated from springs of western Canada. Geochim. Cosmochim. Acta 35, 787–800.

Smith, V.C., Bradford, M.A., 2003. Litter quality impacts on grassland litter decomposition are differently dependent on soil fauna across time. Appl. Soil Ecol. 24, 197–203.

Sterner, R.W., Elser, J.J., 2002. Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere. Princeton University Press, Princeton, New Jersey, USA.

Stout, J.D., Goh, K.M., Rafter, T.A., 1981. Chemistry and turnover of naturally occurring resistant organic compounds in soil. In: Paul, E.A., Ladd, J.N. (Eds.), Soil Biochemistry, vol. 5. Marcel Dekker, New York, pp. 19–24.

Van Kessel, C., Farrell, R.E., Pennock, P.J., 1994. Carbon-13 and nitrogen-15 natural abundance in crop residues and soil organic matter. Soil Sci. Soc. Am. J. 58, 382–389.

Van Veen, J.A., Ladd, J.N., Frissel, M.J., 1984. Modelling C and N turnover through the microbial biomass in soil. Plant Soil 76, 257–274.

Voroney, R.P., Winter, J.P., Beyaert, R.P., 1993. Soil microbial biomass C and N. In: Carter, M.R. (Ed.), Soil Sampling and Methods of Analysis. Canadian Society of Soil Science, Lewis, Chelsea, pp. 277–286.

Wedin, D.A., Tieszen, L.L., Dewey, B., Pastor, J., 1995. Carbon isotope dynamics during grass decomposition and soil organic matter formation. Ecology 76, 1383–1392.