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Microbial community utilization of added carbon substrates in response to long-term carbon input manipulation

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Abstract

The chemical composition and quantity of plant inputs to soil are primary factors controlling the size and structure of the soil microbial community. Little is known about how changes in the composition of the soil microbial community affect decomposition rates and other ecosystem functions. This study examined the degradation of universally ^{13}C-labeled glucose, glutamate, oxalate, and phenol in soil from an old-growth Douglas-fir (*Pseudotsuga menziesii*)—western hemlock (*Tsuga heterophylla*) forest in the Oregon Cascades that has experienced 7 y of chronic C input manipulation. The soils used in this experiment were part of a larger Detritus Input and Removal Treatment experiment and have received normal C inputs (control), doubled wood inputs, or root and litter input exclusion (no inputs). Soil from the doubled wood treatment had a higher fungal:bacterial ratio, and soil from the no inputs treatment had a lower fungal:bacterial ratio, than the control soil. Differences in the utilization of the compounds added to the field-manipulated soils were assessed by following the ^{13}C tracer into microbial biomass and respiration. In addition, ^{13}C-phospholipid fatty acids (PLFA) analysis was used to examine differential microbial utilization of the added substrates. Glucose and glutamate were metabolized similarly in soils of all three litter treatments. In contrast, the microbial community in the double wood soil respired more added phenol and oxalate, whereas microbes in the no inputs soil respired less added phenol and oxalate, than the control soil. Phenol was incorporated primarily into fungal PLFA, especially in soil of the double wood treatment. The addition of all four substrates led to enhanced degradation of soil organic matter (priming) in soils of all three litter treatments, and was greater following the addition of phenol and oxalate as compared to glucose and glutamate. Priming was greater in the no inputs soil as compared to the control or doubled wood soils. These results demonstrate that altering plant inputs to soil can lead to changes in microbial utilization of C compounds. It appears that many of these changes are the result of alteration in the size and composition of the microbial community.

Keywords: ^{13}C; Bacteria; Decomposition; Forest soil; Fungi; Microbial community; Phospholipid fatty acids (PLFA); Priming

1. Introduction

The size and composition of the soil microbial community is a function of net primary production, plant carbon (C) allocation, rhizosphere activity, and litter substrate quality (Smith and Paul, 1990; Fisk and Fahey, 2001; Myers et al., 2001), and is controlled through complex interactions with plants (Zak et al., 2000; Bohlen et al., 2001; Butler et al., 2004). Changes in atmospheric CO_2 concentration and nitrogen (N) deposition rates alter both the quality and quantity of above- and belowground plant litter inputs to soil (Aber et al., 1993; Canadell et al., 1996), which in turn can affect belowground microbial community structure and function (Phillips et al., 2002; Frey et al., 2004; Waldrop et al., 2004). Understanding the mechanisms controlling belowground C processes is useful in predicting future changes in soil C stores in response to climate and land-use change (Pendall et al., 2004).

Altering root and coarse woody debris (CWD) inputs to soil is one method to examine the feedbacks between plants, microbes, and soil organic matter (SOM) dynamics (Nadelhoffer et al., 2004). In a Douglas-fir forest, 7 y of CWD additions and litter and root exclusion have produced significant changes in annual soil CO_2 efflux (Sulzman et al., 2005). Similar results have been reported

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for a deciduous forest (Bowden et al., 1993). Soil solution chemistry was also altered by changes in detrital inputs. Lajtha et al. (2005) showed that 7 y of CWD addition caused an increase in dissolved organic matter (DOM) fluxes from the organic layer to the mineral soil. These differences remained at 30 cm, but had disappeared by 100 cm (Lajtha et al., 2005). Although the quantity of DOM entering the mineral soil was higher following the addition of CWD, the chemistry of the soil solution leaving the organic horizon was the same, suggesting microbial degradation of labile components of CWD before soil solution enters the mineral soil (Lajtha et al., 2005; Yano et al., 2005). Neither the addition of CWD nor root and litter exclusion altered the size of the microbial biomass in the mineral soil (Spears et al., 2003; Brant et al., 2006). Root and litter exclusion did alter the microbial community composition, however, resulting in a loss in fungal biomass and a change in the soil bacterial community structure (Brant et al., 2006).

Although soil respiration, an indicator of overall microbial activity, can be modeled on an ecosystem-scale using soil temperature and moisture (e.g., Andrén et al., 1999; Sulzman et al., 2005), to achieve process-level understanding of decomposition we must know something about microbial community composition (Schimel, 1995). By understanding the ecological controls of microbial community composition, and the role of microbial groups in the decomposition of different organic compounds, we hope to gain a more mechanistic view of litter decomposition and belowground ecosystem function.

Litter is composed of a complex mixture of compounds including recalcitrant polymeric compounds, such as lignin and cellulose, which are broken down into smaller units by exoenzymes. Only a relatively small group of microorganisms, such as fungi and actinomycetes, produce these enzymes. Fungi are responsible for nearly all of the extracellular enzyme production necessary to degrade lignin (Wagner and Wolf, 1999). Therefore, changes in the composition of the ligninolytic portion of the community are likely to have an effect on overall community function (Schimel and Gulledge, 1998). However, structurally distinct microbial communities can have virtually identical C utilization patterns (Buyer and Drinkwater, 1997; Waldrop et al., 2000) as a result of the functional diversity of soil microorganisms and the functional redundancy of soil ecosystems (Ekschmitt and Griffiths, 1998).

Some recently developed techniques allow examination of the linkages between soil microbial community structure and function. One technique involves the addition of ^{13}C-labeled substrates that resemble different components of plant inputs, such as simple sugars, cellobiose, and phenolic compounds. By adding these compounds to soils in the laboratory, and tracking their incorporation into microbial phospholipid fatty acids (PLFA), it is possible to examine how distinct microbial communities process different C compounds (Phillips et al., 2002; DeForest et al., 2004; Waldrop and Firestone, 2004; Waldrop et al., 2004). In addition, by tracking the incorporation of ^{13}C into lipid biomarkers it is possible to determine which members of the microbial community are involved in the decomposition of specific organic substrates.

The addition of both simple and complex organic substrates to soil has been shown to result in a short-term change in the turnover of native SOM, termed the 'priming effect' (Kuzyakov et al., 2000). Substrate additions can lead to either an increase (positive priming) or a decrease (negative priming) in SOM decomposition compared to unamended soil. The priming effect has long been observed; however, little is known about its underlying mechanisms. A recent review found a larger positive priming effect in nutrient poor soils as well as following the addition of more complex substrates (Fontaine et al., 2003). Positive priming of SOM has been shown to occur in laboratory incubations with the addition of various simple organic compounds, plant litter, and root rhizodeposition in a range of soils (Kuzyakov and Cheng, 2001; Hamer and Marschner, 2002, 2005; Waldrop and Firestone, 2004). A recent study has also shown priming as a result of 7 y of litter addition to forest soils at a field scale (Sulzman et al., 2005).

The objective of this study was to determine whether 7 y of CWD addition and root and litter exclusion in an old-growth Douglas-fir forest has altered the ability of the soil microbial community to mineralize a variety of ^{13}C-labeled substrates (glucose, glutamate, oxalate, and phenol). The added substrates are products of plant litter degradation, components of root exudates, or produced by fungi. Coarse woody debris additions have led to an increase in the size of the fungal community and root exclusion has decreased the size of the fungal community. We hypothesized that these changes in fungal:bacterial ratio would, in turn, alter the utilization of more recalcitrant C compounds, such as phenol, whereas the organisms in all litter treatments would be able utilize simple compounds, such as glucose and glutamate, in a similar manner. We also hypothesized that the addition of C compounds would lead to a positive priming effect. The priming effect was expected to be larger in the no inputs soil as a result of its more recalcitrant SOM. Differences in microbial utilization of C compounds were assessed by following the ^{13}C tracer into microbial biomass and respiration, as well examining differences in growth yield efficiency. In addition, microbial groups involved in the degradation of these compounds were identified by following the ^{13}C-label into microbial PLFA.

2. Materials and methods

2.1. Study site

Plant litter inputs have been manipulated at the Detritus Input and Removal Treatment (DIRT) plots in the H.J. Andrews Experimental Forest (HJA) in Oregon (44°15'N,

122°10'W, 531 m elevation) since 1997. Mean annual temperature at the headquarters site of HJA is 8.7 °C (1973–2002) and mean annual precipitation during the same period is 2370 mm y^{-1}, mostly as rain. In general, over 70% of the precipitation occurs during a "wet season", between November and March. Nitrogen deposition to this area is 1.6 kg N ha^{-1} y^{-1} (Vanderbilt et al., 2003). The DIRT site was established in an undisturbed old-growth Douglas-fir (*Pseudotsuga menziesii* (Mirbel) Franco)—western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) stand. Other important tree species at the site include western red cedar (*Thuja plicata* Donn ex D. Don) and vine maple (*Acer circinatum* Pursh). Soils are derived from volcanic parent materials and have been classified as coarse loamy mixed mesic Typic Hapludands (Dixon, 2003).

In 1997, the three litter manipulation treatments used in this study were established as part of a larger inter-site comparison looking at plant controls on SOM formation and transformation over decadal time-scales (Nadelhoffer et al., 2004). The plots at this site are 10 m × 15 m, with three replicates of each treatment. To double the mass of woody debris in the forest floor of double wood (DW) plots, a mix of extremely decomposed woody debris and the chips of large pieces of intact Douglas-fir, with a ratio of decomposed woody debris to intact woody debris of 4:1, are added every other year. The rate of addition is 576 g C m^{-2} y^{-1}. Above- and belowground litter inputs are excluded in the no inputs (NI) plots. Belowground inputs are excluded by trenching the perimeter to 1 m depth, inserting a 10 mil (0.025 cm) thick polyethylene sheet along the bottom and sides of the trench, then back-filling the trenches. Aboveground litter inputs are excluded with 1-mm mesh screens. The control (CO) plots have received normal plant inputs.

2.2. Soil incubations

Soil cores (0–10 cm) were collected from the three replicates of each litter manipulation treatment in September 2003. The soil cores were then composited by treatment to yield one sample for each treatment. The soils were brought back to the laboratory, sieved to 2 mm, and stored at 4 °C until use. Before the incubations began, the soils were brought up to −33 kPa (50% gravimetric water content) and pre-incubated at 22 °C for 5 d.

One of four substrates was added to 12 replicates of each of the three soils, for a total of 144 samples. Fifty μg C g^{-1} soil (~10% of the microbial biomass C) of universally ^{13}C-labeled glucose, glutamate, oxalate, or phenol were added to each 25-g sample of soil in 2 mL of deionized H_2O. Ninety-nine atom% ^{13}C substrates were diluted with unlabeled compounds, such that 22 atom% ^{13}C substrates were added to soils. Two mL of deionized H_2O was added to a second set of samples as a control. The soils were incubated at 22 °C for 14 d in 0.5-L mason jars. Respiration was measured every 6 h for the first 3 d, and then 5, 7, 10, and 14 d after substrate addition. During the first 3 d of the incubation, jars were capped for 6 h with airtight lids fitted with a septum; for all subsequent samplings, the jars were capped for approximately 24 h prior to gas sampling. When uncapped, jars were covered with Parafilm (Pechiney Plastic Packaging, Chicago, IL) to prevent drying. Respiration was measured on three randomly selected replicates of each treatment, as well as the unamended control. At each sampling time, two gas samples were collected from each replicate using airtight plastic syringes (Becton-Dickinson, Franklin Lakes, NJ), and injected into pre-evacuated 10 and 3-mL Vacutainers (Becton-Dickinson, Franklin Lakes, NJ). The 3-mL sample was analyzed for total CO_2 concentration by gas chromatography (Carle Series 100 Analytical GC, Loveland, CO), and the 10-mL sample was analyzed for $\delta^{13}C$ using a PDZ Europa 20/20 isotope ratio mass spectrometer (IRMS; Cheshire, UK). Three randomly selected replicates of each treatment were destructively sampled at 2, 4, 8, and 14 d for microbial biomass carbon (MBC) and PLFA analysis.

2.3. Soil respiration

The percentage CO_2–C coming from the added substrate was calculated with a mixing model as in Waldrop and Firestone (2004)

$$\%C_{sub} = \left[\frac{(\delta_C - \delta_T)}{(\delta_C - \delta_S)}\right] 100, \quad (1)$$

where δ_C is the $\delta^{13}C$ value of the respired CO_2 from the control (no added substrate) soils, δ_T is the $\delta^{13}C$ respired CO_2 in the treated soils, and δ_S is the $\delta^{13}C$ of the labeled substrate. The increase in SOM-C utilization following substrate addition (priming) was calculated as the increase in total soil respiration following substrate addition minus the amount of C respired from the added substrate; this was expressed as a percentage where 100% represents a doubling of SOM-C respiration.

^{13}C–CO_2 evolution kinetics were modeled with single- and double-exponential models using experimental curve-fitting software (SigmaPlot, Systat Software Inc., Point Richmond, CA). Equations for the two models were as follows:

$$\text{Single Exponential Model: } CO_2 - C = A_1\left(1 - e^{-k_1 t}\right), \quad (2)$$

$$\text{Double Exponential Model: } CO_2 - C = A_1\left(1 - e^{-k_1 t}\right) + A_2\left(1 - e^{-k_2 t}\right). \quad (3)$$

2.4. Microbial biomass

Soil was analyzed for MBC immediately following gas sampling. The fumigation–extraction procedure of Vance et al. (1987) was used, as modified by Bruulsema and Duxbury (1996) for ^{13}C analysis. Briefly, a 10 g (dry wt.)

sample of soil was placed on a shaker for 1 h with 30 mL of 0.05 M K_2SO_4 and subsequently filtered through Whatman #40 filter papers. Another sample was fumigated for 24 h with ethanol-free chloroform and extracted in the same way. Three aliquots (0.75 mL) of each K_2SO_4 extract were pipetted onto acetone-rinsed tin squares (37 mm × 37 mm) (Environmental Microanalysis, Manchester, MA), and dried at 60 °C for 2 h after the addition of each aliquot. The tin squares containing the dried extracts were balled up and analyzed for total C and $\delta^{13}C$ using a PDZ Europa 20/20 IRMS. A K_C of 0.45 was used to convert chloroform flush C values into MBC (Wu et al. 1990). The following equation was used to determine the $\delta^{13}C$ value of MBC

$$\delta^{13}C_{MBC} = \frac{\left(\delta^{13}C_{fum} \times C_{fum} - \delta^{13}C_{unfum} \times C_{unfum}\right)}{C_{fum} - C_{unfum}}, \quad (4)$$

where C_{fum} and C_{unfum} refer to the mass of C extracted from the fumigated and the non-fumigated samples, respectively, and $\delta^{13}C_{fum}$ and $\delta^{13}C_{unfum}$ refer to their corresponding $\delta^{13}C$ values. The unfumigated samples were also used to look at the quantity and $\delta^{13}C$ of dissolved organic carbon (DOC). The percentage of MBC and DOC from the added substrate was calculated using Eq. (1), with the exception that the $\delta^{13}C$ of unamended SOM was assumed to be the $\delta^{13}C$ of preincubation SOM.

Microbial growth yield efficiency (GYE) is the fraction of substrate C assimilated by the microbial community that is incorporated into MBC versus being respired as a byproduct of energy production. GYE was calculated using one of the four techniques outlined by Frey et al. (2001)

$$GYE = \frac{dB_C}{\left(dB_C + \sum CO_2\text{–}C\right)}, \quad (5)$$

where dB_C is the amount of ^{13}C-substrate incorporated into MBC and ΣCO_2-C is the cumulative substrate lost during respiration. This method assumes that the sum of biomass C and cumulative substrate respired equals the total amount of substrate utilized. This method does not account for metabolite production or for biomass that is consumed by grazers (Frey et al., 2001).

2.5. PLFA analysis

Lipids were extracted immediately after sampling from 2 g (dry wt.) of soil using the modified Bligh and Dyer method (Bligh and Dyer, 1959; White and Ringelberg, 1998), in which soils were incubated in a 2:1:0.8 solution of methanol, chloroform, and phosphate buffer. The soil extracts were filtered and the chloroform phases collected. Phospholipids were separated from glycolipids and neutral lipids using 3-mL Supelclean LC–Si solid phase extraction columns (Supelco Park, Bellefonte, PA). Phospholipids were saponified and methylated to fatty-acid methyl esters (FAME). FAME were then run twice, first on an Agilent 6890 gas chromatograph (GC; Agilent Technologies, Palo Alto, CA) equipped with a flame ionization detector (FID) and an HP Ultra-2 column for total PLFA community identification, and next on an Agilent 6890 GC equipped with an HP Innowax-2 column connected to a Europa ORCHID on-line combustion interface attached to a Europa 20/20 IRMS to measure the $\delta^{13}C$ of PLFA. Peaks were identified based on comparing retention times with known standards. Concentration of each PLFA was obtained by comparing peak areas with a 13:0 FAME standard curve. The $\delta^{13}C$ of each individual lipid was corrected for the methyl group added during transesterification (Abraham et al., 1998).

Specific PLFA were used as biomarkers of the relative abundance and $\delta^{13}C$ (weighted average) of various taxonomic groups (White and Ringelberg, 1998; Waldrop et al., 2000; Zak et al., 2000): Gram-positive bacteria (i15:0, a15:0, i16:0, i17:0, and a17:0), Gram-negative bacteria (cy17:0, cy19:0, and 18:1ω7c), fungi (18:2ω6c), and actinomycetes (10Me16:0). A ratio of the fungal PLFA to the sum of the bacterial PLFA was calculated as an index of the relative proportions of fungi and bacteria for comparing effects of added substrates and soils. This type of ratio has been used by many others (e.g., Phillips et al., 2002; Bååth and Anderson 2003; McMahon et al., 2005), but is not directly comparable to fungal:bacterial ratios calculated with other methods, such as substrate induced respiration or microscopic counts (Bardgett et al., 1996; Lin and Brookes, 1999; Bååth and Anderson 2003). The total amount of isotope incorporated into fungal PLFA was divided by the amount of ^{13}C in bacterial PLFA to produce a fungal:bacterial incorporation ratio (Waldrop et al., 2004). Relative substrate incorporation was calculated by multiplying the mol% of each PLFA taxonomic biomarker by the percentage of that biomarker coming from the added substrate, which was calculated using a mixing model.

2.6. Statistical analysis

Relative abundances (mol %) for all 40 PLFA identified were used to examine community differences among treatments. Microbial community structure was examined by non-metric multidimensional scaling (NMS; Kruskal, 1964; Mather, 1976) using the PC-ORD software package (MjM software, Gleneden Beach, OR). The medium setting of 'autopilot' mode in PC-ORD was used. Final ordinations were rotated to maximize environmental variables represented and separation of treatments on axes 1 and 2. Statistical differences between whole PLFA profiles were assessed using multi-response permutation procedures (MRPP; Mielke and Berry, 2001) in PC-ORD.

The statistical significance of the effects of substrate and sampling day on PLFA taxonomic groups in each soil, as well as their interaction, was analyzed with repeated measures analysis of variance (ANOVA) using PROC MIXED (SAS version 8.1, SAS Institute, Inc., Cary, NC). In instances where the interaction between treatment and day was not significant ($p > 0.05$), the interaction was

removed and the analysis was repeated using data from all four sampling days. When ANOVA resulted in a p-value <0.05, pre-planned comparisons among the four substrates were made using orthogonal contrasts with the Tukey-Kramer procedure to correct for family wise error rates. Differences between the three soils through time following substrate addition were analyzed using the same method. When only day 2 data were examined, one-way ANOVA was used with the Tukey-Kramer procedure to correct for family wise error rates (Splus version 6.1, Insightful Corp., Seattle, WA).

3. Results

3.1. Microbial community composition

None of the substrates affected MBC in any of the soils during the whole incubation, although the NI soil had lower MBC than the CO and DW soils (Table 1). At all four sampling days the three soils had significantly different PLFA profiles (data not shown; multi-response permutation procedures (MRPP), $p<0.05$). The added substrates had no effect on the PLFA profile in any of the three different soils (MRPP; $p>0.05$), except for the DW soil (MRPP; $p=0.02$) on day 2 and both the CO (MRPP; $p=0.01$) and DW (MRPP; $p<0.001$) soils on day 14 of the incubation. These changes were slight and there were no significant effects of the substrates on the relative abundance of any of the taxonomic markers during the course of the incubation (data not shown; ANOVA, $p>0.05$). Fig. 1 is a non-metric multi-dimensional scaling (NMS) plot showing the microbial community differences among the soils with data from all four substrates on day 2, as it is representative of soil differences throughout the incubation. The three soils are distinct along axis 2. The three soils differ in the relative abundance of fungi, with the DW soil having the largest and the NI soil the smallest fungal population (Table 1). The same trend is also seen in the fungal:bacterial ratio among soils. The DW soil has the lowest relative abundance of the three different bacterial taxonomic markers compared with the other two soils (Table 1).

As there was no significant effect of any of the substrates on the MBC or the relative abundance of any of the taxonomic markers, the data from all four substrates was combined to look at changes in the microbial community during the 14-d incubation (Table 1). In the DW treatment, the relative abundance of fungi and Gram-negative bacteria generally decreased throughout the incubation, while the relative abundance of Gram-positive bacteria increased. Accordingly, the fungal:bacterial ratio decreased through time. The relative abundance of actinomycetes fluctuated during the incubation with no particular pattern and the MBC stayed relatively constant during the incubation, although there was a slight decline in MBC between 8 and 14 d. The MBC in the CO soil was inconsistent during the incubation, being high at 2 and 8 d, and low at 4 and 14 d (Table 1). Changes in microbial community structure in the CO soil followed a similar pattern to that in the DW soil, although there was less of a decline in the relative abundance of fungi and a larger increase in gram-positive bacteria (Table 1). In the NI soil, MBC as well as the relative abundance of fungi remained constant throughout the experiment. Similar trends were found in the Gram-negative and Gram-positive abundances as seen in the DW and CO soils, however the changes through time in Gram-negative bacteria were not significant.

3.2. Substrate utilization

The four substrates were respired by the microbial community at different rates (Fig. 2). A large portion of the

Table 1
Soil differences in summed relative abundance of taxonomic biomarkers and MBC over the 14-d incubation

Treatment	Day	Fungi (mol%)	Gram-negative bacteria (mol%)	Gram-positive bacteria (mol%)	Actinomycetes (mol%)	Fungal:bacterial ratio	MBC ($\mu g\,C\,g^{-1}$ soil)
Double wood	2	**5.15 (0.21)**^{a}	**21.41 (0.38)**^{ab}	12.75 (1.02)^{a}	**6.59 (0.21)**^{ab}	**0.106 (0.005)**^{ab}	292.5(14.8)^{ab}
	4	**4.93 (0.25)**^{a}	**21.80 (0.25)**^{a}	13.06 (0.86)^{a}	**7.03 (0.18)**^{ab}	**0.100 (0.006)**^{a}	292.9 (21.5)^{ab}
	8	**4.51 (0.09)**^{a}	**20.61 (0.18)**^{b}	16.05 (0.43)^{b}	**6.59 (0.05)**^{a}	**0.090 (0.002)**^{a}	332.5 (9.8)^{a}
	14	**4.09 (0.13)**^{b}	**20.28 (0.27)**^{b}	15.80 (0.68)^{b}	**7.03 (0.11)**^{b}	**0.082 (0.002)**^{b}	248.6 (10.1)^{b}
Control	2	3.68 0.12)^{ab}	23.23 (0.48)^{a}	15.49 (1.14)^{ab}	8.12 (0.12)	0.071 (0.002)^{ab}	306.3 (6.9)^{ab}
	4	4.01 (0.20)^{a}	23.03 (0.09)^{a}	15.18 (0.40)^{a}	7.95 (0.13)	0.078 (0.004)^{a}	263.8 (18.4)^{bc}
	8	3.67 (0.10)^{b}	22.80 (0.37)^{ab}	15.86 (0.67)^{a}	8.19 (0.10)	0.070 (0.002)^{b}	334.0 (11.6)^{a}
	14	3.28 (0.14)^{b}	21.86 (0.18)^{b}	17.50 (0.46)^{b}	8.30 (0.17)	0.063 (0.003)^{b}	265.3 (11.5)^{c}
No inputs	2	**1.90 (0.06)**	24.22 (0.41)	13.38 (0.93)^{a}	**7.43 (0.15)**	**0.035 (0.001)**	**192.6 (15.3)**
	4	**1.98 (0.08)**	23.58 (0.27)	15.19 (0.68)^{b}	**7.21 (0.11)**	**0.037 (0.001)**	**203.5 (12.2)**
	8	**1.99 (0.21)**	22.56 (0.36)	16.41 (0.54)^{b}	**7.37 (0.10)**	**0.038 (0.004)**	**225.5 (16.6)**
	14	**1.76 (0.07)**	**22.76 (0.26)**	15.60 (0.54)^{b}	**7.46 (0.17)**	**0.033 (0.001)**	**194.6 (5.6)**

Numbers show the mean of all substrates ($n=12$) with standard error in parentheses. Different letters indicate a significant difference (ANOVA; $p<0.05$) in the relative abundance of a particular biomarker over time. Numbers in bold are significantly (ANOVA; $p<0.05$) different from the Control soil on a specific sampling date.

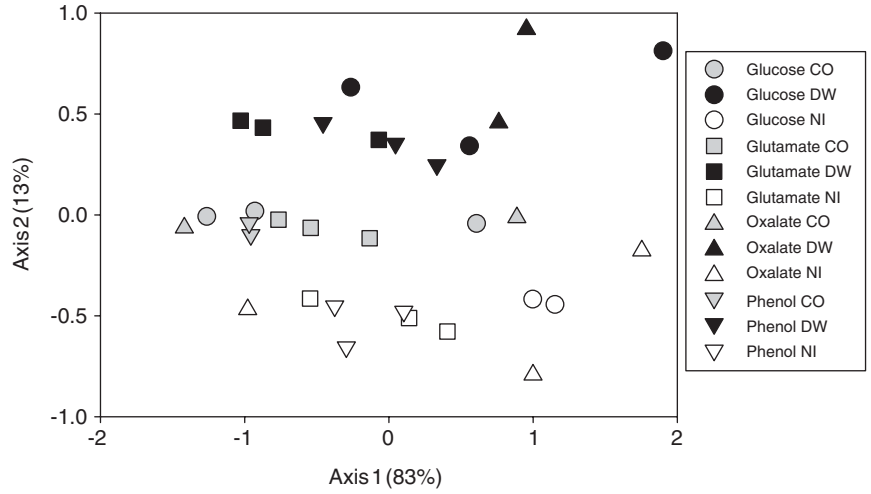

Fig. 1. NMS of PLFA relative abundance on day 2 of the incubation. There was no difference in PLFA profiles by substrate, so all substrates are shown for each soil. White symbols are for NI soils, gray symbols are for Control soils, and black symbols are for DW soils. Circles are for glucose, squares are for glutamate, triangles are for oxalate, and upside-down triangles represent phenol. R^2 axis 1 = 0.83, axis 2 = 0.13.

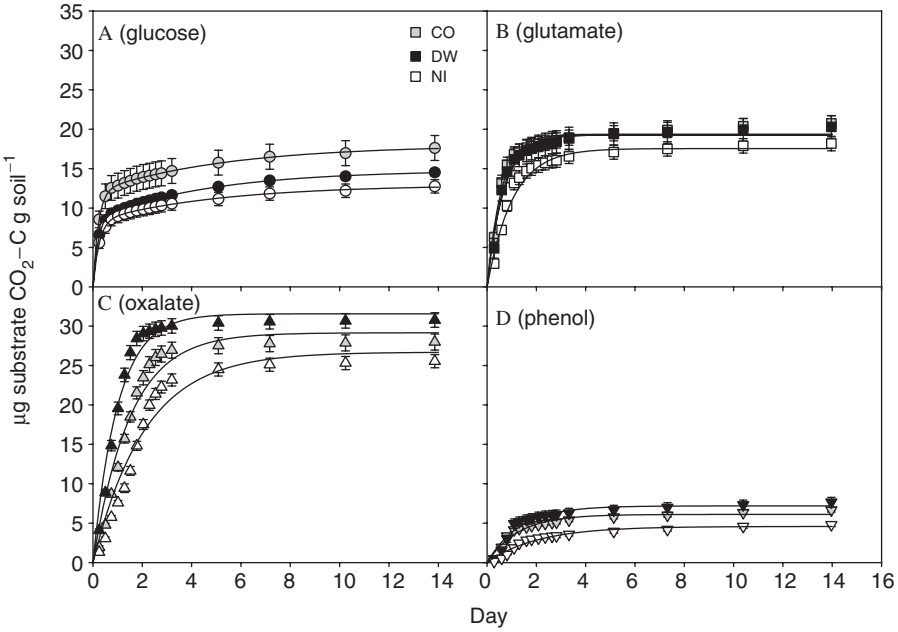

Fig. 2. Cumulative substrate respiration for all four substrates. Each point represents the mean ($n = 3$) amount of substrate respired at the 16 sampling points during the incubation. Error bars are ±1 S.E. of the mean. Curves are best-fit double-exponential models for (A) glucose respiration (circles), and single-exponential models for (B) glutamate (squares), (C) oxalate (triangles), and (D) phenol respiration (upside-down triangles). White symbols are for NI soils, gray symbols are for Control soils, and black symbols are for DW soils.

respiration of all substrates occurred in the first 2 d following substrate addition. In both unamended soils (data not shown) and the field (Sulzman et al., 2005), respiration rates were significantly lower from the NI soils, and significantly higher from the DW soils as compared to the CO soils. The kinetics of oxalate, phenol, and glutamate respiration were well explained using a single first-order exponential model (Fig. 2). On the other hand, glucose respiration was poorly explained using a single first-order model ($R^2 < 0.70$) and a double exponential model was necessary to sufficiently explain the data. In the glutamate, oxalate, and phenol addition treatments at least 90% of the respired substrate was respired during the first 4 d of incubation (Fig. 2). Contrary to the hypothesis that readily available substrates would be respired quickly, only 83% of the total substrate respired was respired after 4 d following glucose addition.

3.2.1. Glucose

The amount of glucose respired differed among soils of all three litter treatments (Fig. 2). During the 14-d incubation, more glucose was respired from the CO soil

(17.6 μg g^{-1} soil) than either the DW (14.5 μg g^{-1} soil) or NI soil (12.8 μg g^{-1} soil). In all three soils the majority of the glucose respired was respired before 2 d. On day 2, the amount of substrate incorporated into MBC was very high in soils of all three litter treatments, although there was no significant difference among soils (Table 2). The large quantity of substrate incorporated into MBC is shown by an especially high GYE during the first 2 d (Table 2). In all three soils, 70–80% of the substrate utilized by the microbial community was incorporated into cells. The specific substrate respiration rate (mg ^{13}C–CO_2 g^{-1} MBC h^{-1}) was fairly low for glucose given the high rate of substrate incorporation, with no significant differences among soils of the different litter treatments (Table 2). The ratio of fungal:bacterial incorporation of ^{13}C-glucose into PLFA was significantly higher in the DW soil than in either CO or NI soil (Table 2). The quantity of ^{13}C-glucose recovered in the DOC pool after 2 d was significantly affected by litter treatment, with an increase in recovery in the DW (75%) and NI (113%) compared to the CO soil.

3.2.2. Glutamate

The amount of ^{13}C-glutamate respired during the 14-d incubation was similar in soils of all three litter treatments (Fig. 2), with 18.2 μg ^{13}C g^{-1} soil respired from the NI soil compared to 20.7 μg ^{13}C g^{-1} soil from the CO soil and 20.3 μg ^{13}C g^{-1} soil from the DW soil. Approximately 90% of the glutamate respired was respired during the first 3 d of incubation in soils of all three litter treatments. The amount of ^{13}C-glutamate incorporated into MBC during the first 2 d was less than the amount of ^{13}C-glucose incorporated into MBC (Table 2). By the end of the incubation, the CO (9.0 μg ^{13}C g^{-1} soil) and DW (8.6 μg ^{13}C g^{-1} soil) soils incorporated significantly more ^{13}C-glutamate into biomass than the NI soil (6.6 μg ^{13}C g^{-1} soil). Although less ^{13}C-glutamate was incorporated into biomass in the NI soils, there were no soil differences in GYE. Similar to glucose, the amount of ^{13}C-glutamate recovered in DOC was significantly smaller in the CO soil than either NI or DW soil. Glutamate was incorporated into fungal and bacterial lipids at the same proportion in soils of all three litter treatments. The specific substrate respiration rate for the added glutamate was significantly higher in the NI soil, than in either the CO or DW soils.

3.2.3. Oxalate

More oxalate was recovered in microbial respiration than for any of the other substrates (Fig. 2). Oxalate also showed the largest differences in the amount of ^{13}C-substrate respired among the three soils. Microbes in the DW soil respired 31.9 μg ^{13}C g^{-1} soil, compared with 29.1 μg ^{13}C g^{-1} soil from the CO soil and 26.5 μg ^{13}C g^{-1} soil from the NI soil after 14 d. Very little oxalate was incorporated into MBC (Table 2), and there was significantly less oxalate incorporated into MBC in the NI soil than in either the CO or DW soils. The large difference in incorporation into biomass led to a significantly lower GYE in NI soils compared with CO and DW soils. The amount of ^{13}C-oxalate recovered in DOC was significantly higher in the NI soils than in either the CO or DW soils. The specific substrate respiration was highest in NI soil and lowest in DW soil at 2 d.

3.2.4. Phenol

Less ^{13}C-phenol was respired during the 14 d than the other three substrates (Fig. 2). After 14 d, more ^{13}C was respired from the DW soil (7.7 μg ^{13}C g^{-1} soil), than either the CO (6.1 μg ^{13}C g^{-1} soil) or NI (4.5 μg ^{13}C g^{-1} soil) soil. There was significantly more ^{13}C-phenol incorporation into MBC of DW compared to the other treatments and more

Table 2
Incorporation of the four substrates into microbial pools and utilization parameters on Day 2 of the incubation

Substrate	Treatment	MBC from substrate (μg ^{13}C g^{-1} soil)	Substrate in DOC (μg ^{13}C g^{-1} soil)	Growth yield efficiency	Specific substrate respiration (mg ^{13}C-CO_2 g^{-1} MBC h^{-1})	Fungal:bacterial ^{13}C incorporation ratio
Glucose	Double wood	33.3 (6.8)	2.86 (0.02)[a]	0.76 (0.19)	0.146 (0.053)	0.35 (0.02)[a]
	Control	45.6 (2.6)	1.63 (0.14)[b]	0.77 (0.06)	0.100 (0.005)	0.24 (0.02)[b]
	No inputs	45.0 (1.0)	3.48 (0.20)[a]	0.82 (0.03)	0.124 (0.028)	0.22 (0.01)[b]
Glutamate	Double wood	7.64 (0.71)[a]	0.56 (0.02)[a]	0.30 (0.03)	0.140 (0.015)[a]	0.26 (0.01)
	Control	7.99 (0.14)[a]	0.38 (0.01)[b]	0.31 (0.01)	0.124 (0.010)[a]	0.25 (0.01)
	No inputs	5.85 (0.23)[b]	0.52 (0.02)[a]	0.28 (0.02)	0.265 (0.051)[b]	0.23 (0.01)
Oxalate	Double wood	1.44 (0.19)[a]	0.41 (0.01)[a]	0.047 (0.006)[a]	0.369 (0.015)[a]	0.20 (0.07)
	Control	1.22 (0.25)[a]	0.48 (0.08)[a]	0.049 (0.010)[a]	1.11 (0.27)[b]	0.24 (0.03)
	No inputs	0.15 (0.08)[b]	2.36 (0.06)[b]	0.009 (0.005)[b]	2.42 (0.19)[c]	0.18 (0.02)
Phenol	Double wood	4.18 (0.13)[a]	0.32 (0.01)[a]	0.42 (0.03)	0.098 (0.011)[a]	0.35 (0.01)[a]
	Control	2.93 (0.06)[b]	0.11 (0.01)[b]	0.38 (0.01)	0.055 (0.009)[b]	0.23 (0.01)[b]
	No inputs	1.84 (0.01)[c]	0.14 (0.01)[b]	0.37 (0.02)	0.164 (0.029)[c]	0.22 (0.02)[b]

Numbers are the mean ($n = 3$) with standard errors in parentheses. Different letters denote a significant (ANOVA; $p < 0.05$) difference among the three soils within each substrate.

^{13}C-phenol recovered in DOC of DW as compared to other treatments (Table 2). There was also a significantly higher ratio of ^{13}C incorporation into fungal lipids than bacterial lipids in the DW soils. Although the DW microbial community was able to incorporate more ^{13}C phenol into biomass there was no difference in GYE across litter treatments during the first 2 d of the incubation (Table 2); however, there was a significant difference in specific substrate respiration among soils of all three litter treatments on day 2 of the incubation, with respiration highest in NI and lowest in CO soil.

3.3. Priming

The addition of all four substrates led to positive priming in soils of all three litter treatments (Fig. 3). Generally, cumulative priming, which is the total amount of excess SOM degraded during the whole incubation, was largest through the first 3 d and subsequently declined after that. In the glucose treatment, cumulative priming was largest in the CO soil, with a maximum of 120%. After the first 3 d cumulative priming decreased exponentially in all soils; by the end of the incubation, priming in response to glucose addition was largest in the NI soil (18.6%), followed by CO (9.8%) and DW (9.8%). Similarly, following glutamate addition the priming effect was largest in the CO soil over the first 3 d. Cumulative priming decreased during the incubation, although the decrease was less rapid than in the glucose addition. By the end of the experiment, the CO (20.9%) and NI (21.1%) soils had a larger cumulative priming than DW (9.1%) soil. The shape of the cumulative priming curves following oxalate addition was similar to those for glucose and glutamate, with a peak in priming around 1 d, followed by a decline. By the end of the incubation NI (30.1%) had a larger cumulative priming effect than CO (13.7%) or DW (4.9%) soil. Lastly, the shape of the cumulative priming curve following phenol addition was a bit different from that of the other treatments. In the CO soil, priming followed an exponential decline over the course of the incubation. However, in the DW and NI soils cumulative priming declined through the first 2 d and then began to increase until day 14. At day 14 the priming effect in the NI (26.4%) soil was larger than in CO (20.8%) and DW (13.3%) soils.

3.4. ^{13}C incorporation into PLFA

3.4.1. Glucose

Fig. 4 shows the δ^{13}C value for different taxonomic groups following glucose addition. In soils of all three litter treatments all of the taxonomic biomarkers were well labeled by day 2, and there was little change in the δ^{13}C of these lipids during the 14-d incubation. There was a larger δ^{13}C for the fungal biomarker in the NI soil. These soils have a smaller fungal biomass (Table 1); the same amount of incorporation into a smaller biomass could yield a larger δ^{13}C value. In fact, when the δ^{13}C values of the taxonomic biomarkers are relativized by the mol%, there was more glucose incorporation into the fungal lipids in the DW soil than either the CO or NI soils (Fig. 5). If the fungal value from the NI soil is ignored, the incorporation into the other taxonomic groups had a similar pattern and δ^{13}C value in the soils of all three litter treatments.

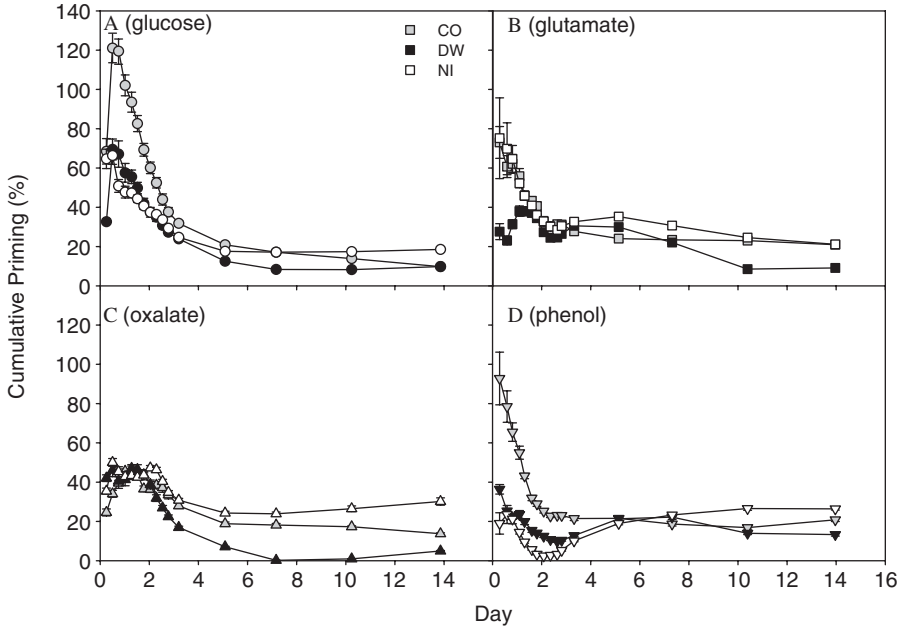

Fig. 3. Cumulative priming during the 14-d incubation for (A) glucose respiration (circles), (B) glutamate (squares), (C) oxalate (triangles), and (D) phenol (upside-down triangles) additions. Symbols show the mean priming ($n = 3$) with error bars ± 1 S.E. White symbols are for NI soils, gray symbols are for Control soils, and black symbols are for DW soils.

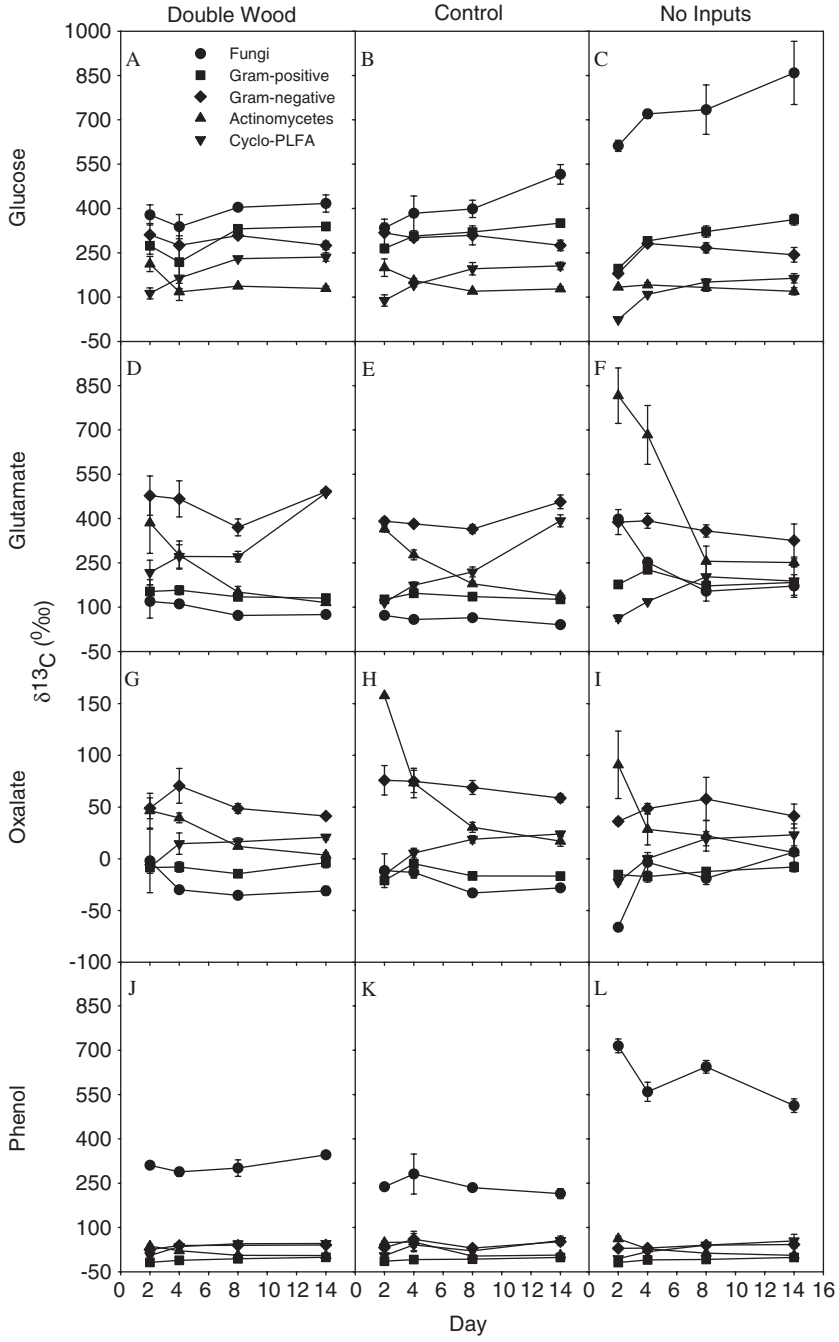

Fig. 4. The $\delta^{13}C$ incorporation into five taxonomic biomarkers through time in the three soils following substrate addition. Each symbol is the weighted mean $\delta^{13}C$ ($n = 3$) with error bars ± 1 S.E. Each panel represents a separate substrate for each of the three litter-amended soils. Symbols are for PLFA biomarkers: fungi (circles), Gram-negative bacteria (diamonds), Gram-positive bacteria (squares), actinomycetes (triangles), cyclo-PLFA (upside-down triangles).

3.4.2. Glutamate

The pattern of ^{13}C-glutamate incorporation into lipids was somewhat different than glucose (Fig. 4). The actinomycete biomarker had a large $\delta^{13}C$ by day 2 of the incubation, and this value declined during the rest of the incubation. The Gram-negative, Gram-positive, and fungal biomarker $\delta^{13}C$ remained relatively stable throughout the 14-d incubation, while the $\delta^{13}C$ of the cyclo lipids increased in soils of all three litter treatments. In the NI soil, the incorporation into actinomycete biomarker was much higher than in the CO and DW soils by day 2. Even with the smaller MBC there was a higher relative incorporation of glutamate into actinomycete lipids on day 2 of the incubation (Fig. 5). Otherwise, the soils of the three treatments responded in a similar fashion, with the exception of higher incorporation into fungal

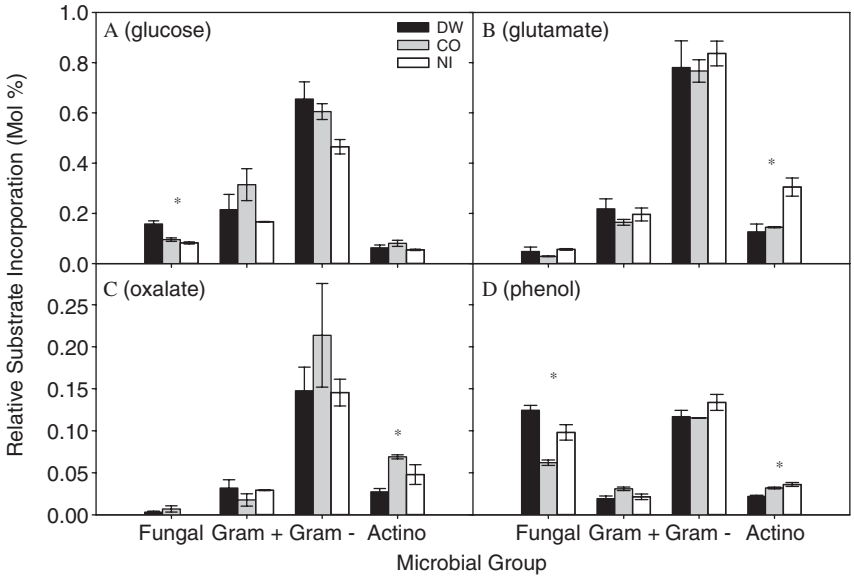

Fig. 5. Relative $\delta^{13}C$ incorporation into PLFA biomarkers of day 2 of the incubation. Each bar is the mean ($n = 3$) incorporation ± 1 S.E. Stars indicate a significant difference (ANOVA; $p < 0.05$) among the three soils for each substrate. Note the different y-axis scale for panels C and D. White bars are for NI soils, gray bars are for Control soils, and black bars are for DW soils.

lipids in the NI soil, followed by a decline as the incubation progressed.

3.4.3. Oxalate

Following oxalate addition there was a much smaller amount of ^{13}C incorporation into lipids than with the addition of the glucose or glutamate (Fig. 4). Moreover, the $\delta^{13}C$ oxalate incorporated into soils of all three litter treatments was the same, instead of higher in the NI soil as with the other substrates (Fig. 4). In all three soils the actinomycete biomarker $\delta^{13}C$ declined throughout the incubation, while the $\delta^{13}C$ of the cyclo PLFA increased (Fig. 4). The $\delta^{13}C$ of the actinomycete markers was higher on day 2 in the CO and NI soil, although these differences were gone by day 8. When ^{13}C incorporation of oxalate was relativized by mol%, there was more incorporation into CO lipids than DW lipids on day 2 (Fig. 5). The $\delta^{13}C$ of the other microbial biomarkers remained relatively stable during the incubation, with little difference among soils.

3.4.4. Phenol

Incorporation of ^{13}C-phenol into lipids was much different than the other three substrates (Fig. 4). There was fairly high incorporation of phenol into the fungal biomarker, with little or no incorporation into any other lipids at any time during the experiment. The $\delta^{13}C$ of the fungal biomarker in NI soil was higher than in the soils of CO or DW treatments, but when relativized by mol%, there was more incorporation into the fungal community in the DW soil than into the CO soil, with intermediate incorporation in the NI soil (Fig. 5). There was lower relative incorporation of phenol into the actinomycete biomarker in the DW soil than in the CO and NI soils (Fig. 5).

4. Discussion

4.1. Soil microbial community dynamics

Substrate additions did not alter the PLFA profile or MBC in any of the three soils examined (Fig. 1, Table 1). We added a small quantity of C compared with most other studies looking at the response of the soil microbial community to substrate additions in order to avoid a large increase in biomass and the selection of certain portions of the microbial community. In a study where a model root exudate was added to soil at different concentrations, no detectable changes in the soil PLFA profile were found until rates of addition exceeded 375 μg C g^{-1} d^{-1} in a 14-d experiment (Griffiths et al., 1999), well above the rate of addition in this study. However, Baudoin et al. (2003) added 100 μg C g^{-1} d^{-1} of a model root exudates consisting of simple sugars and amino acids over 14 d, and observed changes in genetic profile and substrate utilization. In another study with one-time C addition to soil from an oak woodland, the addition of 400 μg C g^{-1} soil of vanillin was found to change the PLFA profile, although no change was seen with similar addition rates of both simple and complex sugars (Waldrop and Firestone, 2004). Falchini et al. (2003) reported changes in microbial community composition following the addition of 726 μg C g^{-1} oxalate and glutamate, but not glucose. Thus, studies that have observed changes in microbial community structure following substrate addition all added significantly more C that was added in this study. In addition many of those

studies used different methods to assess the microbial community, which may be more sensitive to small changes in microbial community composition than the method we used.

In all three of our soils, the Gram-negative bacteria generally decreased during the 14 d incubation and the Gram-positive bacteria increased (Table 1). Very few studies have reported changes in the microbial community through time following substrate addition. After 14 d Griffiths et al. (1999) found increases in the relative abundance of PLFA from Gram-positive bacteria, Gram-negative bacteria, and actinomycetes, although each of these taxonomic groups responded differently to the substrate additions in that study. Waldrop and Firestone (2004) found an increase in Gram-positive and some Gram-negative PLFA 9 d after substrate addition to an oak woodland soil. Neither Waldrop and Firestone (2004) nor Griffiths et al. (1999) reported changes through time following substrate addition.

4.2. Substrate utilization

The percentage of added substrate respired during the incubation was consistent with other studies using similar substrates (Shen and Bartha, 1996; Hamer and Marschner, 2002, 2005; Falchini et al., 2003; Waldrop et al., 2004), although most of these studies added between 10 and 100 times the amount of C added in this study. The amount of added substrate respired differed by both soil and substrate. As hypothesized, the amount of substrate respired following glucose and glutamate additions was similar in soils of all three litter treatments, with the exception that more glucose was respired from the CO soil. All of the difference in the amount of ^{13}C-glucose respired from the CO soil compared with the DW soil occurred in the first 12 h of incubation (3.0 μg ^{13}C g^{-1} soil at 12 h, 3.1 μg ^{13}C g^{-1} soil at 14 d), after which the two soils behaved similarly (Fig. 2). With oxalate and phenol, there was more ^{13}C-substrate respired from the DW and CO soils than from the NI soil. The DW and CO soils are more fungally dominated, and the DW soil would be expected to have a larger community of wood-decomposing fungi (Table 1). White-rot fungi have the ability to degrade simple phenolic compounds (Bending and Read, 1997) and are major producers of oxalate in soil (Dutton and Evans, 1996). Thus, an increase in this type of fungi could lead to increased oxalate production, and a greater ability of the microbial community to degrade these types of compounds.

The cumulative respiration of oxalate, phenol, and glutamate was fit well using a single first-order exponential model. The ability of a single exponential model to explain the substrate respiration curves suggests that there is one easily mineralizable pool of substrate C following addition of these three substrates, most of which is respired during the first 3 d of incubation. Glucose, on the other hand, continued to be respired at a low rate throughout the incubation (Fig. 2). In addition, the GYE following glucose addition was very high at day 2 of the incubation (Table 2) and decreased as the incubation progressed (data not shown). The GYE in our soils following glucose addition fall at the high end of the published values, but are not unreasonable (Frey et al., 2001, Thiet et al. 2006). Nguyen and Guckert (2001) reported that in soils with C limitation, glucose was taken into the cell and may have been converted to storage compounds rather than used for growth or respired as CO_2 during the short-term. This stored glucose could then be utilized later in the incubation, leading to the appearance of multiple C pools derived from the added glucose.

Two days following the addition of all four substrates, the ratio of fungal to bacterial substrate incorporation was at least 0.2 in soils of all three litter treatments (Table 2). This number is greater than the fungal:bacterial ratio of the soils themselves (Table 1), suggesting that fungi were more actively utilizing the added substrates than were bacteria. There were no differences among soils in fungal:bacterial ^{13}C-incorporation following glutamate and oxalate addition, but the DW soil fungi responded to glucose and phenol addition.

On day 2 of the incubation, there was no difference in glucose incorporation into MBC in the three soils (Table 2). There was also no soil difference in GYE or specific substrate respiration, implying a similar ability of microbial communities in the three soils to utilize glucose. The microbes in the DW soil incorporated a larger percentage of ^{13}C-glucose into fungal lipids than in the other two treatments. Thus, the small observed differences in ^{13}C-respiration and microbial incorporation could be due to differences in microbial community structure, especially fungal:bacterial ratio. A higher fungal:bacterial ratio has been shown to lead to increased incorporation of cellobiose into fungal lipids, with little or no changes in the amount of cellobiose respired (Phillips et al., 2002). The distinct microbial communities in these three soils had slight differences in their incorporation of glucose and small differences in glucose respiration, suggesting the functional equivalence of these microbial communities in degrading simple sugars (Waldrop and Firestone, 2004).

The amount of glutamate respired did not vary much among soils, with slightly less ^{13}C respired from the NI soil (Fig. 2). Even though the soils are similar in terms of the amount of substrate respired, there is some evidence of differential processing in the NI soil: There was more ^{13}C incorporated into actinomycete PLFA in the NI soil (Fig. 4).

Very little of the added oxalate was incorporated into either MBC (Table 2) or PLFA (Fig. 4), with less substrate incorporated in the MBC in the NI soil. Even though the GYE for the whole soil microbial community was very low after oxalate addition, some microorganisms were able to grow on oxalate as evidenced by incorporation of ^{13}C into microbial lipids. The taxonomic groups most able to use oxalate were Gram-negative bacteria and actinomycetes.

Most oxalate-degrading bacteria isolated from terrestrial ecosystems fall into these two broad categories (Sahin, 2003). The microbial community in the NI soil seemed to have trouble degrading oxalate when compared to those in CO and DW soils given the low substrate incorporation into MBC and high specific respiration rate (Table 2). Most oxalate in soil is either produced by fungi (Dutton and Evans, 1996) or excreted by plant roots (Bertin et al., 2003). The NI plots have a small fungal biomass as well as no plant inputs to soil and could have a smaller population of oxalate-degrading bacteria as a result.

It appears that only a small portion of the microbial population was able to utilize phenol, given the low respiration rate and small incorporation into MBC. However, the few microorganisms that were able to utilize the substrate seemed to have no problem incorporating phenol into microbial biomass, given the GYE value of roughly 0.4 for this substrate. Phenol is toxic to most organisms at high concentrations. Following phenol addition, there was either a toxic effect, a period of enzyme induction, or growth of phenol-degrading microorganisms as evidenced by the S-shaped ^{13}C respiration curve, especially in the NI soil (Fig. 2). It appears that if there was a toxic effect of phenol, it was fairly small and transient, as there was no difference in the PLFA profile or MBC by day 2 in the phenol addition experiment compared with the other substrates. Fungi were responsible for most of the phenol degradation in this ecosystem, as well as some Gram-negative bacteria and actinomycetes (Figs. 4 and 5). White-rot fungi are the major phenol oxidase producers and phenol degraders in soil (Dix and Webster, 1995), although Gram-negative bacteria, Gram-positive bacteria, and actinomycetes with the ability to degrade phenol have been isolated from soils (McCarthy and Williams, 1992; Koutny et al., 2003).

4.3. Priming

All four of the added substrates induced positive priming in each of the three soils (Fig. 3). Many other studies have shown positive priming in response to the addition of the same simple C compounds used in this study (e.g., Dalenberg and Jager, 1989; Shen and Bartha, 1996; Hamer and Marschner, 2002, 2005; Falchini et al., 2003; Waldrop and Firestone, 2004), although most of those have added significantly more C than we did. One study that added simple C substrates in lower concentrations than we used (11.3–34 μg C g soil^{-1}) found positive priming over 3 d (De Nobili et al., 2001). In the only study we found examining priming effects in response to phenol addition (Shen and Bartha, 1996), a large transient priming effect was found to occur during the first 15 d of incubation, similar to the results in this experiment. Those authors claimed their findings were the result of turnover of organisms killed by phenol addition, even at quantities below those added in this experiment. As noted above, if there was a toxic effect of phenol in our study it was undetectable at 2 d in either MBC or PLFA profiles, and was probably small at the low concentration of phenol added in this experiment.

The largest priming effect was found during the first 2 d of the incubation, which was also the period of highest substrate respiration (Figs. 2 and 3). This result has been reported in other studies as well (Bell et al., 2003; Falchini et al., 2003; Hamer and Marschner, 2005). It has been hypothesized that the priming effect in response to C addition is caused by the turnover of microbial biomass C, rather than the decomposition of native SOM (Dalenberg and Jager, 1989; Wu et al., 1993; De Nobili et al., 2001; Bell et al., 2003 ; Thiet et al. 2006). These studies have shown that the turnover of MBC is the source of positive priming following the addition of various simple C compounds and plant residues in quantities above and below those added in this study. An alternative theory presented by De Nobili et al. (2001) hypothesized that the soil microbial biomass is in a resting state most of the time, but can be quickly stimulated to turnover endogenous C by the addition of certain trigger molecules. This can result in a large, transient, positive priming effect following the addition of even small amounts of simple C compounds.

The largest positive priming effect was found in the NI soil (Fig. 3). The NI soils are expected to have mostly recalcitrant C, as they have not received new C inputs for over 7 y. Fontaine et al. (2003) reported that nutrient-poor soils generally have a larger priming effect than nutrient-rich soil, and that the addition of more recalcitrant compounds generally produces a larger priming effect than simple C compounds. The two compounds used in this study least available for microbial growth (oxalate and phenol) indeed induced a larger priming effect than the two more labile compounds as found in other studies, although these differences were fairly small.

4.4. Conclusions

Microbial utilization of all four substrates differed among all three litter treatments. The most pronounced differences were the utilization of phenol and oxalate, where the amount of ^{13}C-respired from the DW soil was larger than the CO soil, which was larger than the amount respired from the NI soil. In soils of all three litter treatments most of the phenol incorporation was into the fungal PLFA. As we hypothesized, it appears that many of the differences in substrate metabolism are the result of an increase in the fungal:bacterial ratio in the DW soil. The larger fungal community in the DW soil is able to degrade more of this substrate than the more bacterially dominated communities of the other soils. It also appears that a larger portion of the fungal community in the DW soil is able to process phenol, as evidenced by an increase in the ratio of ^{13}C-fungal:^{13}C-bacterial substrate incorporation following phenol addition. More research is needed in order to identify if the DIRT litter manipulation treatments lead to changes in fungal community structure, as well as which

fungi are important in the degradation of phenolic C compounds.

In the future, changes in atmospheric chemistry and N deposition could lead to long-term changes in the quality and quantity of plant inputs to soil. Understanding the structural and mechanistic changes altered plant inputs produce in the soil microbial community may allow us to better understand and predict how these changes might influence ecosystem functioning and soil C sequestration. Seven years of chronic changes in plant inputs to an old-growth forest have led to changes in the structure of the microbial community, as well as its ability to degrade a variety of C compounds. Many of these changes appear to be the result of a shift in the fungal community. More work is needed to assess how the functional changes observed in laboratory incubations translate into actual ecosystem functioning.

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References

Aber, J.D., Magill, A., Boone, R., Melillo, J.M., Steudler, P., Bowden, R., 1993. Plant and soil responses to chronic nitrogen additions at the Harvard Forest, Massachusetts. Ecological Applications 3, 156–166.

Abraham, W., Hesse, C., Pelz, O., 1998. Ratios of carbon isotopes in microbial lipids as an indicator of substrate usage. Applied and Environmental Microbiology 64, 4202–4209.

Andrén, O., Brussaard, L., Clarholm, M., 1999. Soil organism influence on ecosystem-level processes bypassing the ecological hierarchy? Applied Soil Ecology 11, 177–188.

Bååth, E., Anderson, T.H., 2003. Comparision of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA based techniques. Soil Biology & Biochemistry 35, 955–963.

Bardgett, R.D., Hobbs, P.J., Frostegård, Å., 1996. Changes in soil fungal:bacterial biomass ratios following reductions in the intensity of management of an upland grassland. Biology and Fertility of Soils 22, 261–264.

Baudoin, E., Benizri, E., Guckert, A., 2003. Impact of artificial root exudates on the bacterial community structure in bulk soil and maize rhizosphere. Soil Biology & Biochemistry 35, 1183–1192.

Bell, J.M., Smith, J.L., Bailey, V.L., Bolton, H., 2003. Priming effect and C storage in semi-arid no-till spring crop rotations. Biology and Fertility of Soils 37, 237–244.

Bending, G.D., Read, D.J., 1997. Lignin and soluble phenolic degradation by ectomycorrhizal and ericoid mycorrhizal fungi. Mycological Research 101, 1348–1354.

Bertin, C., Yang, X.H., Weston, L.A., 2003. The role of root exudates and allelochemicals in the rhizosphere. Plant and Soil 256, 67–83.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37, 911–917.

Bohlen, P.J., Groffman, P.M., Driscoll, C.T., Fahey, T.J., Siccama, T.G., 2001. Plant–soil–microbial interactions in a northern hardwood forest. Ecology 82, 965–978.

Bowden, R.D., Nadelhoffer, K.J., Boone, R.D., Melillo, J.M., Garrison, J.B., 1993. Contributions of aboveground litter, belowground litter, and root respiration to total soil respiration in a temperature mixed hardwood forest. Canadian Journal of Forest Research 23, 1402–1407.

Brant, J.B., Myrold, D.D., Sulzman, E.W., 2006. Root controls on soil microbial community structure in forest soils. Oecologia, in press, doi:10.1007/s00442-006-0402-7.

Bruulsema, T.W., Duxbury, J.M., 1996. Simultaneous measurement of soil microbial nitrogen, carbon, and carbon isotope ratio. Soil Science Society of America Journal 60, 1787–1791.

Butler, J.L., Bottomley, P.J., Griffith, S.M., Myrold, D.D., 2004. Distribution and turnover of recently fixed photosynthate in ryegrass rhizospheres. Soil Biology & Biochemistry 36, 371–382.

Buyer, J.S., Drinkwater, L.E., 1997. Comparison of substrate utilization assay and fatty acid analysis of soil microbial communities. Journal of Microbiological Methods 30, 3–11.

Canadell, J.G., Pitelka, L.F., Ingram, J.S.I., 1996. The effects of elevated CO_2 on plant-soil carbon below-ground: a summary and synthesis. Plant and Soil 187, 391–400.

Dalenberg, J.W., Jager, G., 1989. Priming effect of some organic additions to C-14-labeled soil. Soil Biology & Biochemistry 21, 443–448.

De Nobili, M., Contin, M., Mondini, C., Brookes, P.C., 2001. Soil microbial biomass in triggered into activity by trace amounts of substrate. Soil Biology & Biochemistry 33, 1163–1170.

DeForest, J.L., Zak, D.R., Pregitzer, K.S., Burton, A.J., 2004. Atmospheric nitrate deposition and the microbial degradation of cellobiose and vanillin in a northern hardwood forest. Soil Biology & Biochemistry 36, 965–971.

Dix, N.J., Webster, J., 1995. Fungal Ecology. Chapman and Hall, London 549 pp.

Dixon, J.J., 2003. Applying GIS to soil geomorphologic landscape mapping in the Lookout Creek valley, Western Cascades, Oregon. M.S. Crop and Soil Science, Oregon State University, 78 pp.

Dutton, M.V., Evans, C.S., 1996. Oxalate production by fungi: its role in pathogenicity and ecology in the soil environment. Canadian Journal of Microbiology 42, 881–895.

Ekschmitt, K., Griffiths, B.S., 1998. Soil biodiversity and its implications for ecosystem functioning in a heterogeneous and variable environment. Applied Soil Ecology 10, 201–215.

Falchini, L., Naumova, N., Kuikman, P.J., Bloem, J., Nannipieri., P., 2003. CO_2 evolution and denaturing gradient gel electrophoresis profiles of bacterial communities in soil following addition of low molecular weight substrates to simulate root exudation. Soil Biology & Biochemistry 35, 775–782.

Fisk, M.C., Fahey, T.J., 2001. Microbial biomass and nitrogen cycling responses to fertilization and litter removal in young northern hardwood forests. Biogeochemistry 53, 201–223.

Fontaine, S., Mariotti, A., Abbadie, L., 2003. The priming effect of organic matter: a question of microbial competition? Soil Biology & Biochemistry 35, 837–843.

Frey, S.D., Gupta, V.V.S.R., Elliott, E.T., Paustian, K., 2001. Protozoan grazing affects estimates of carbon utilization efficiency of the soil microbial community. Soil Biology & Biochemistry 33, 1759–1768.

Frey, S.D., Knorr, M., Parrent, J.L., Simpson, R.T., 2004. Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. Forest Ecology and Management 196, 159–171.

Griffiths, B.S., Ritz, K., Ebblewhite, N., Dobson, G., 1999. Soil microbial community structure: effects of substrate loading rates. Soil Biology & Biochemistry 31, 145–153.

Hamer, U., Marschner, B., 2002. Priming effects of sugars, amino acids, organic acids and catechol on the mineralization of lignin and peat. Journal of Plant Nutrition and Soil Science 165, 261–268.

Hamer, U., Marschner, B., 2005. Priming effects in different soil types induced by fructose, alanine, oxalic acid and catechol additions. Soil Biology & Biochemistry 37, 445–454.

Koutny, M., Ruzicka, J., Chlachula, J., 2003. Screening for phenol-degrading bacteria in the pristine soils of south Siberia. Applied Soil Ecology 23, 79–83.

Kruskal, J.B., 1964. Nonmetric multidimentional scaling: a numerical method. Psychometrika 29, 115–129.

Kuzyakov, Y., Cheng, W., 2001. Photosynthesis controls of rhizosphere respiration and organic matter decomposition. Soil Biology & Biochemistry 33, 1915–1925.

Kuzyakov, Y., Friedel, J.K., Stahr, K., 2000. Review of mechanisms and quantification of priming effects. Soil Biology & Biochemistry 32, 1485–1498.

Lajtha, K., Crow, S.Y., Yano, S.S., Kaushal, E.W., Sulzman, J.D.H., Spears, 2005. Detrital controls on soil solution N and dissolved organic matter in soils: a field experiment. Biogeochemistry 76, 261–281.

Lin, Q., Brookes, P.C., 1999. Comparison of selective inhibition and biovolume measurements of microbial biomass and its community structure in unamended, ryegrass-amended, fumigated and pesticide-treated soils. Soil Biology & Biochemistry 31, 1999–2014.

Mather, P.M., 1976. Computation Methods of Multivariate Analysis in Physical Geography. Wiley, London 532 pp.

McCarthy, A.J., Williams, S.T., 1992. Actinomycetes as agents of biodegradation in the environment—a review. Gene 115, 189–192.

McMahon, S.K., Williams, M.A., Bottomley, P.J., Myrold, D.D., 2005. Dynamics of microbial communities during decomposition of carbon-13 labeled ryegrass fractions in soil. Soil Science Society of America Journal 69, 1238–1247.

Mielke Jr., P.W., Berry, K.J., 2001. Permutation Methods: A Distance Function Approach. Springer, New York 325 pp.

Myers, R.T., Zak, D.R., White, D.C., Peacock, A., 2001. Landscape-level patterns of microbial community composition and substrate use in upland forest ecosystems. Soil Science Society of America Journal 65, 359–367.

Nadelhoffer, K.J., Boone, R.D., Bowden, R.D., Canary, J.D., Kaye, J., Micks, P., Ricca, A., Aitkenhead, J.A., Lajtha, K., McDowell, W.H., 2004. The DIRT experiment: litter and root influences on forest soil organic matter stocks and function. In: Foster, D., Aber, J. (Eds.), Forests in Time: The Environmental Consequences of 1000 Years of Change in New England. Yale University Press, New Haven, pp. 300–315.

Nguyen, C., Guckert, A., 2001. Short-term utilisation of C-14-glucose by soil microorganisms in relation to carbon availability. Soil Biology & Biochemistry 33, 53–60.

Pendall, E., Bridgham, S., Hanson, P.J., Hungate, B.A., Kicklighter, D.W., Johnson, D.W., Law, B.E., Luo, Y.Q., Megonigal, J.P., Olsrun, M., Ryan, M.G., Wan, S.Q., 2004. Below-ground process responses to elevated CO_2 and temperature: a discussion of observations, measurement methods, and models. New Phytologist 162, 311–322.

Phillips, R.L., Zak, D.R., Holmes, W.E., White, D.C., 2002. Microbial community composition and function beneath temperate trees exposed to elevated atmospheric carbon dioxide and ozone. Oecologia 131, 236–244.

Sahin, N., 2003. Oxalotrophic bacteria. Research in Microbiology 154, 399–407.

Schimel, J.P., 1995. Ecosystem consequences of microbial diversity and community structure. In: Chapin, III, F.S., Korner, C. (Eds.), Arctic and Alpine Biodiversity: Patterns, Causes and Ecosystem consequences. Springer, New York, pp. 239–254.

Schimel, J.P., Gulledge, J., 1998. Microbial community structure and global trace gases. Global Change Biology 4, 745–758.

Shen, J., Bartha, R., 1996. Priming effect of substrate addition in soil-based biodegradation tests. Applied and Environmental Microbiology 62, 1428–1430.

Smith, J.L., Paul, E.A., 1990. The significance of soil microbial biomass estimations. In: Bollag, J., Stotzky, G. (Eds.), Soil Biochemistry. Mercel Dekker, New York, pp. 357–393.

Spears, J.D.H., Holub, S.M., Harmon, M.E., Lajtha, K., 2003. The influence of decomposing logs on soil biology and nutrient cycling in an old-growth mixed coniferous forest in Oregon, USA. Canadian Journal of Forest Research 33, 2193–2201.

Sulzman, E.W., Brant, J.B., Bowden, R.D., Lajtha, K., 2005. Contribution of aboveground litter, belowground litter, and rhizosphere respiration to total soil CO_2 efflux in an old growth coniferous forest. Biogeochemistry 73, 231–256.

Thiet, R.K., Frey, S.D., Six, J., 2006. Do growth yield efficiencies differ between soil microbial communities differing in fungal:bacterial ratios? Reality check and methodological issues. Soil Biology & Biochemistry, in press, doi:10.1016/j.soilbio.2005.07.010.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology & Biochemistry 19, 703–707.

Vanderbilt, K.L., Lajtha, K., Swanson, F.J., 2003. Biogeochemistry of unpolluted forest watersheds in Oregon Cascades: temporal patterns of precipitation and stream nitrogen fluxes. Biogeochemistry 62, 87–117.

Wagner, G.H., Wolf, D.C., 1999. Carbon transformations and soil organic matter formation. In: Sylvia, D.M., Fuhrmann, J.J., Hartel, P.G., Zuberer, D.A. (Eds.), Principles and Applications of Soil Microbiology. Prentice Hall, Upper Saddle River, NJ, pp. 218–258.

Waldrop, M., Balser, T., Firestone, M., 2000. Linking microbial community composition to function in a tropical soil. Soil Biology & Biochemistry 32, 1837–1846.

Waldrop, M., Firestone, M.K., 2004. Microbial community utilization of recalcitrant and simple carbon compounds: impact of oak-woodland plant communities. Oecologia 138, 275–284.

Waldrop, M.P., Zak, D.R., Sinsabaugh, R.L., 2004. Microbial community response to nitrogen deposition in northern forest ecosystems. Soil Biology & Biochemistry 36, 1443–1451.

White, D.C., Ringelberg, D.B., 1998. Signature biolipid biomarker analysis. In: Burlage, R.S., Burlage, R.S., Atlas, R., Stahl, D., Geesey, G., Sayler, G. (Eds.), Techniques in Microbial Ecology. Oxford University Press, New York, pp. 255–272.

Wu, J., Joergensen, R.G., Pommerening, B., Chaussod, R., Brookes, P.C., 1990. Measurement of soil microbial biomass C by fumigation—extraction—an automated procedure. Soil Biology & Biochemistry 22, 1167–1169.

Wu, J.G., Brookes, P.C., Jenkinson, D.S., 1993. Formation and destruction of microbial biomass during the decomposition of glucose and ryegrass in soil. Soil Biology & Biochemistry 25, 1435–1441.

Yano, Y., Lajtha, K., Sollins, P., Caldwell, B.A., 2005. Chemistry and dynamics of dissolved organic matter in a temperate coniferous forest on andic soils: effects of litter quality. Ecosystems 8, 286–300.

Zak, D.R., Pregitzer, K.S., Curtis, P.S., Holmes, W.E., 2000. Atmospheric CO_2 and the composition and function of soil microbial communities. Ecological Applications 10, 47–59.