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Microbial community utilization of recalcitrant and simple carbon compounds: impact of oak-woodland plant communities

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Abstract Little is known about how the structure of microbial communities impacts carbon cycling or how soil microbial community composition mediates plant effects on C-decomposition processes. We examined the degradation of four ^{13}C-labeled compounds (starch, xylose, vanillin, and pine litter), quantified rates of associated enzyme activities, and identified microbial groups utilizing the ^{13}C-labeled substrates in soils under oaks and in adjacent open grasslands. By quantifying increases in non-^{13}C-labeled carbon in microbial biomarkers, we were also able to identify functional groups responsible for the metabolism of indigenous soil organic matter. Although microbial community composition differed between oak and grassland soils, the microbial groups responsible for starch, xylose, and vanillin degradation, as defined by ^{13}C-PLFA, did not differ significantly between oak and grassland soils. Microbial groups responsible for pine litter and SOM-C degradation did differ between the two soils. Enhanced degradation of SOM resulting from substrate addition (priming) was greater in grassland soils, particularly in response to pine litter addition; under these conditions, fungal and Gram + biomarkers showed more incorporation of SOM-C than did Gram – biomarkers. In contrast, the oak soil microbial community primarily incorporated C from the added substrates. More ^{13}C (from both simple and recalcitrant sources) was incorporated into the Gram – biomarkers than Gram + biomarkers despite the fact that the Gram + group generally comprised a greater portion of the bacterial biomass than did markers for the Gram – group. These experiments begin to identify components of the soil microbial community responsible for decomposition of different types of C-substrates. The results demonstrate that the presence of distinctly different plant communities did not alter the microbial community profile responsible for decomposition of relatively labile C-substrates but did alter the profiles of microbial communities responsible for decomposition of the more recalcitrant substrates, pine litter and indigenous soil organic matter.

Keywords Microbial community composition · ^{13}C-phospholipid fatty acid analysis · Soil carbon cycling · Enzyme activities

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Introduction

Plant communities can influence associated soil microbial communities through the types and amounts of C and nutrient inputs (e.g. plant litter, exudates, epiphyte litter, animal and atmospheric depositional inputs) and by altering the temperature and water content of the soil (Eviner 2001; Myers et al. 2001; Stark and Firestone 1996). In the oak-grassland ecosystems of California, oak trees occur at varying densities in extensive areas of grassland. Differences in soil organic matter (SOM) quantity and quality and nutrient availability create 'islands of fertility' in soils under oak canopies that may be expected to support different microbial communities than the adjacent open grassland soils (Dahlgren et al. 1997; Herman et al. 2003; Jackson et al. 1990). The input of oak litter into soils under oak canopies may be expected to produce a microbial community more able to degrade complex C, because litter inputs from oak contain more lignin and tannin than grassland litter (Deschaseaux and Ponge 2001; Lopez Llorca and Olivares Bernabeu 1997).

Soil microbes have commonly been viewed as black boxes into which organic carbon flows and is converted into CO_2 or biomass. Flow rates through this black box are

affected by temperature and moisture, but in some cases microbial community composition, size, and physiology also may affect the degradation rates of carbon substrates primarily through shifts in enzymatic capacity (Schimel and Gulledge 1998; Wall and Moore 1999; Zogg et al. 1997). Process rates have however been measured with no accompanying detectable change in community composition (Balser 2002; Houston et al. 1998). Alternatively, differences in microbial communities may lead to no change in function (Finlay et al. 1997). This lack of correspondence between community composition and function may occur because measures of whole microbial community composition do not identify groups of organisms of critical importance to the process being measured.

It has been hypothesized that changes in macromolecular C (e.g. lignin and tannin) degrading functional groups are more critical to ecosystem processes than changes in simple C (e.g. sugars and amino acids) degrading functional groups because of lower species richness of microbes with the enzymatic capacity for macromolecular C degradation (Schimel and Gulledge 1998). Microbial community composition can now be linked to the utilization of carbon substrates by supplying ^{13}C-labeled substrates and tracking the isotope into microbial phospholipid biomarkers (PLFAs) thereby defining functional groups involved in in situ decomposition processes. Herein, we define a functional group as those PLFA biomarkers that incorporate specific organic substrates.

When organic substrates are added to soil, microbial activity is commonly stimulated and a "priming effect" (PE) may occur in which both the added substrate and indigenous soil organic materials (SOM) are degraded. This priming effect can substantially increase the degradation rate of indigenous SOM, potentially altering C storage and the C balance of entire ecosystems (Cheng 1999). Very little is known about the microbial groups that may be involved in this long-observed but poorly understood phenomenon.

Microbial groups responsible for the degradation of a range of carbon substrates were identified by applying ^{13}C-labeled substrates (xylose, starch, vanillin, and pine needles) and then quantifying the appearance of the ^{13}C in PLFA biomarkers. Similarly, microbial groups responsible for the decomposition of non-^{13}C-enriched soil organic material were assessed. The impacts of plants on the identity of the microbial groups responsible for decomposition were determined by comparing the ^{13}C dynamics in microbial communities occurring in soils under oak canopies to those in immediately adjacent open grassland plots.

Materials and methods

Soils

Fresh grassland and oak soils up to 15-cm deep were collected from Hopland Research and Extension Center in March 2001 and aboveground plant material removed. Oak soils were taken from beneath blue oak (*Quercus douglasii*) canopies, and grassland soils were collected from the open grassland, at least 10 m from the nearest oak tree. *Avena barbata* and *Bromus hordeaceus* were the dominant plant species in the grassland at the time of sampling. Soils were brought back to the laboratory, sieved (2 mm), cleared of any observable roots, and pre-incubated for 5 days at room temperature. Soil C and N were measured using a Carlo Erba NC2100 elemental analyzer. Soil pH, water holding capacity, soil texture, and available P (Bray−1) were measured using standard procedures (Page et al. 1982). Both soils were sandy clay loams of the Sutherlin series.

Soil incubations

We added one of four substrates to three replicate samples of oak and grassland soil. Universally ^{13}C-labeled (90–99 atom %) starch, vanillin, xylose (0.4 mg substrate g soil^{-1}) and air-dried ^{13}C-labeled, finely ground, loblolly pine (*Pinus taeda* L.) litter (12 mg C g soil^{-1}). Pine litter came from Free-Air Carbon dioxide Enrichment (FACE) facility in the Duke University Forest (NC) (δ^{13}C=−43‰) and had a C/N ratio of 73.9±1.1. Pine litter was used as a complex substrate that would require an array of extracellular enzymes for its degradation. We added a larger quantity of pine litter to ensure that ^{13}C would be detected in all soil pools. Starch, vanillin, and xylose C-substrates were added in solution and stirred into the soil to ensure a homogeneous mixture. The pine substrate was added dry and then water was added to the soil and stirred. The control treatment was treated in the same way as the substrate-amended soils, but without the addition of a C source. Soils were maintained at –3.0 MPa water potential by adjusting water content at each sampling date according to water characteristic curves.

Soils were incubated at 20°C for 65 days in 1-l airtight mason jars using lids fit with rubber septa. Soil respiration, the isotope ratio of respired CO_2,and enzyme activities were measured on 2, 9, 19, 42, and 65 days after substrate addition. Headspace CO_2 was sampled through the mason jar lid septa using gas-tight 10 ml plastic syringes (Becton Dickinson). One sample was directly injected into a Shimadzu 14A gas chromatograph with a thermal conductivity detector to measure CO_2 concentration. A second aliquot of the headspace gas was transferred into an evacuated and sealed gas-sampling vial for carbon isotope ratio determination. Carbon isotope ratios of respired CO_2were measured on a Micromass mass spectrometer (Isoprime) with a Micromass trace gas preconcentrator using a Perkin Elmer Headspace sampler HS-40. After each gas sampling, jars were opened and soils were subsampled for enzyme and PLFA analysis.

The percent of the CO_2-C coming from added substrate was calculated as:

$$\%\mathrm{C}_{\mathrm{substrate}} = \left[\frac{(\delta_{\mathrm{C}} - \delta_{\mathrm{T}})}{(\delta_{\mathrm{C}} - \delta_{\mathrm{L}})}\right] \times 100 \quad (1)$$

where δ_C is the δ^{13}C value of the respired CO_2 evolved from the control soils (no substrate added), δ_T is the δ^{13}C respired CO_2 in the treated soil, and δ_L is the δ^{13}C of the labeled substrate. The increase in SOM-C utilization was calculated as the increase in total soil respiration following substrate amendment minus the amount of C respired specifically from the added label expressed as a percent, where 100% is a doubling of SOM-C respiration.

Enzyme assays

We assayed eight carbon degrading enzymes: β−1, 4-glucosidase, cellobiohydrolase, β-xylosidase, N-acetyl-glucosaminidase, galactase, α−1, 4-glucosidase and phenol oxidase. All enzyme assays except phenol oxidase used methylumbellyferyl (MUB)-substrate solutions. One gram of soil was added to 100 ml of a 5 mM pH 8.0

bicarbonate buffer solution, and stirred on a stir plate while sampling with a multichannel pipettor. A 100 µl aliquot of the mixture was added to each lane of a 96-well microplate that contained 100 µl of MUB substrate solution. Controls for fluorescence quenching by soil materials were made by adding 100 µl of increasing concentrations of MUB (0.5 to 2.5 µM) to 100 µl of sample solution in the microplate. Each soil sample had eight replicate enzyme assays and four replicate quenching controls. A Fluorolog 3 spectrofluorometer with a Micromax plate reader (ISA Instruments) was used to measure fluorescence. Excitation was set at 360 nm and emission was measured at 450 nm. Plates were incubated at 27°C for 2.5 h. Emission was measured at the beginning and end of the incubation. Enzyme activities are expressed as mol $h^{-1}g^{-1}$ dry soil.

Phenol oxidase activity was measured using 50 mM L-dihydroxyphenyalanine (L-DOPA in 5 mM bicarbonate buffer) as substrate. LDOPA solution (100 µl) and 100 µl soil solution (1:100 w/v) were added to wells of a 96-well plate. Controls were made using 100 µl soil solution and 100 µl 5 mM bicarbonate buffer. There were six analytical replicates and two control replicates per sample. The activity was calculated as the increase in absorbance at 469 nm over 1 h using a Spectramax plus spectrophotometer (Molecular Devices). Phenol oxidase activity is expressed as mg h^{-1} g^{-1} dry soil.

Microbial community composition

Microbial community composition was measured using phospholipid fatty acid (PLFA) biomarkers on days 2, 9, 19, and 65. Microbial biomass was quantified as the sum total of extracted PLFAs (Frostegård and Bååth 1996). PLFAs were extracted from 5 g of freeze-dried soil (White and Ringleberg 1998) with the following modifications. Instead of using a separating funnel during the initial extraction, we allowed the phases to separate in a glass vial and used suction through a glass pipette to remove the upper aqueous phase; we evaporated solvent using a slow stream of N_2 gas. Extracted phospholipid samples were analyzed using a Hewlett Packard 6890 Gas Chromatograph with a 25 m ×0.2 mm ×0.33 µm Ultra 2 (5%-phenyl)-methylpolysiloxane column (Hewlett Packard) using H_2 as the carrier, N_2 as the make-up gas, and air to support the flame. The GC analyzed a 1 µl injection with a 1:100 split, at an initial temperature of 170°C, ramped to 260°C at 2°C min^{-1} at a constant flow rate of 0.4 ml min^{-1}. Peaks were identified using bacterial fatty acid standards and MIDI peak identification software (MIDI, Newark, DE). Only biomarkers that made up more than 1% of the total biomass were included in community composition analysis.

PLFA biomarkers provide a generally coarse fingerprint of microbial community structure. There are only a few markers for fungi and actinomycetes which can comprise a major portion of the soil microbial biomass. A number of biomarkers are associated with Gram-positive (+) and Gram-negative (−) bacteria. This Gram + and Gram − distinction is based ultimately on cell wall structure and has some phylogenetic significance in that Gram + organisms are generally clustered in phylogenetic displays (Madigan et al. 2003). The Gram +/− categorization functions here primarily as a means of community stratification because there are currently no robust associations known among carbon utilization capacities and Gram reaction. 'Saturated' PLFAs contain no double bonds, and are not specific to any bacterial or fungal group. Biomarker abundances were converted to mol% values and the first two principal components generated by Principal Components Analysis (PCA) of the mol% values were used to create an aggregate index of community characteristics.

Isotope ratios of microbial PLFAs were measured on a Finnigan Delta plus mass spectrometer with a GC/C III interface (Thermo-finnigan) coupled to a HP 5973 GC (Agilent technologies). We corrected the measured isotope ratio for the additional methanol group added during transesterification using the procedure of Abraham et al. (1998). Biomarker-specific utilization of added substrate (pmol biomarker from added substrate) was calculated as a weighted isotope difference:

$$\text{pmol substrate utilized} = \left[\frac{\delta_S - \delta_B}{\delta_S - \delta_L}\right] \times \text{pmols of biomarker} \quad (2)$$

where δ_S is the isotope ratio of the SOM-C, δ_B is the isotope ratio of the biomarker and δ_L is the isotope ratio of the added label. Biomarker-specific utilization of SOM-C was calculated by subtracting the picomols of the individual PLFA biomarker made from added substrate from the absolute increase in biomarker size, assuming that increases in biomarker size must come from substrate or SOM-C. We limited the analysis of ^{13}C-PLFA to the second time point (day 9) because this point had the largest average increase in biomarker abundances over control. We focused on the 17 most abundant biomarkers in the soil sample. In addition, we analyzed ^{13}C-PLFA data from day 65 in the pine treatment where both positive and negative 'priming effects' occurred.

Statistical analysis

We used a two-way Repeated Measures ANOVA to test the effect of soil type (grassland and oak) and treatment (four substrates and control) on microbial community and carbon cycling variables. ^{13}C-PLFA data were analyzed by one-way ANOVA testing the effect of soil type on the incorporation of each label into each specific lipid. We used Principal Components Analysis (PCA; JMP software; SAS Institute) to construct new aggregate variables from multi-component PLFA data sets. Two-way ANOVA was used to analyze the effect of soil and substrate on ^{13}C-PLFA principal component data. These data were not analyzed using Repeated Measures because temporal data were limited to one, or in the case of pine, two, time points. Significant differences from ANOVA (P <0.05) were further analyzed using the Tukey-Kramer HSD test. Substrate respiration and ^{13}C-incorporation data were normalized to the quantity of substrate added. The enzymatic response to substrate addition was calculated as substrate treatment activity minus control activity divided by control soil activity. Regression analysis was performed using the enzymatic response as the independent variable and substrate respiration as the dependent variable (JMP software; SAS Institute).

Results

Soil characteristics

Compared to the grassland soil, the oak soil had more soil carbon (36 vs 12 g C kg^{-1}), nitrogen (2.4 vs 1.2 g N kg^{-1}), and available phosphorus (40 vs 10 mg P kg^{-1}). Microbial biomass (7,777 and 3,420 nmol PLFA kg^{-1}), soil respiration (29 and 15 mg C kg^{-1} dry soil $days^{-1}$), and enzyme activities were also higher in the oak soil than the grassland soil, possibly reflecting the oak soil's higher carbon content (Table 1). On the other hand, specific soil respiration was lower in the oak soil compared to the grassland soil (0.81 vs 1.25 mg C g^{-1} C day^{-1}) Microbial community composition differed between the grassland and oak soils (Fig. 1) due to differences in the relative abundance of all but six PLFA biomarkers. The oak soil had a higher relative abundance of eight biomarkers including both Gram + and Gram – markers. The grassland soil contained a higher relative abundance of two fungal biomarkers (18:3ω6c and 18:2ω6c), the actinomycete biomarker 10ME18:0, and five, primarily Gram +, bacterial biomarkers (Tables 2, 3).

Table 1 Characteristics of oak and grassland. Values are means ± SE (n =3–5). Different superscripts indicate values that are significantly different (P <0.05)soils

	C (g kg^{-1})	N (g kg^{-1})	C/N	Bray P (mg P kg^{-1})	pH	Microbial biomass (nmol PLFA kg^{-1})	Respiration rate (mg C kg^{-1}day^{-1})	Specific respiration rate (mg C g C^{-1}day^{-1})
Oak (*Quercus douglasii*)	36±0.3^{a}	2.4±0.01^{a}	14.6±0.5^{a}	40.1±1.8^{a}	5.4±0.1^{a}	7,777±250^{a}	29±2.0^{a}	0.81±0.1^{b}
Grassland (*Avena barbata*, *Bromus hordeaceus*)	12±0.1^{b}	1.2±0.01^{b}	10.1±0.5^{b}	10.2±0.3^{b}	5.2±0.1^{b}	3,420±154^{b}	15±1.0^{b}	1.25±0.2^{a}

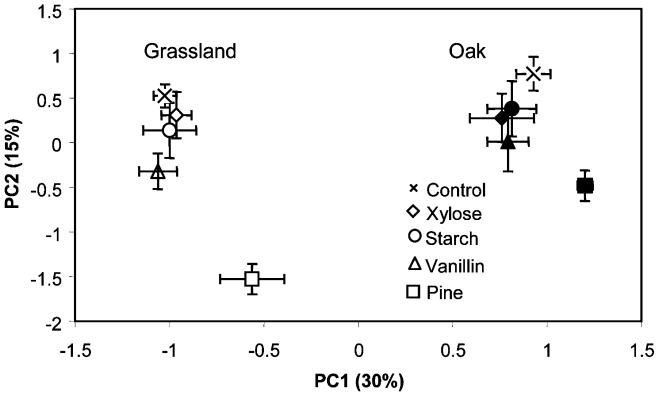

Fig. 1 Microbial community composition represented by the first and second principal components of PLFA mol% data. Oak (*black symbols*) and grassland communities (*white symbols*) differed from one another along both Principal Component 1 (PC1) and PC2 axes. Microbial community composition was altered from the control by pine litter and vanillin addition. Percentages represent the amount of variability explained by the principal component. Values are means ± SE (n =12)

Response to substrate addition

Community composition

Substrate amendments increased microbial biomass by 15–21% compared to the control soils (Table 3); there were no substrate by time interactions for microbial biomass. Overall community composition (e.g. PLFA PC1 or PC2, Fig. 1) was altered by the addition of pine litter and vanillin. Four PLFAs had significant soil by treatment interactions, in which biomarker abundance changed in response to substrate additions in one soil type but not the other (Table 2). The majority of the PLFAs displayed a significant treatment effect, in which pine litter or vanillin addition altered biomarker abundance (Table 3). The addition of xylose or starch did not significantly affect community composition, which is also reflected in the lack of significant changes in biomarker abundances (Tables 2, 3). In general, following pine litter or vanillin additions, the relative abundance of Gram + biomarkers decreased and the relative abundance of saturated (e.g. 14:0, 16:0), and the Gram – biomarkers 18:1ω7c increased (Tables 2, 3).

Pine addition appeared to alter the composition of the community specifically increasing the relative abundance of the saturated biomarkers, two Gram − biomarkers, and the fungal biomarker 18:2ω6c while decreasing the relative abundance of six Gram + biomarkers and Gram – biomarker 16:1ω5c in both soils (Tables 2, 3).

Substrate degradation

There were few differences in xylose, starch, and vanillin degradation either within or between soils. Degradation rates of xylose, starch, and vanillin were faster than pine litter, and were unaffected by plant community and microbial community differences (Fig. 2). On the other

Table 2 Relative abundance of bacterial, fungal, and actinomycete PLFA biomarkers in grassland and oak soils following substrate additions. Letters indicate differences among treatment means (n =12). Values without letters indicate a non-significant treatment and soil by treatment interaction

	Grassland					Oak				
	Control	Pine	Xylose	Vanillin	Starch	Control	Pine	Xylose	Vanillin	Starch
Saturated	Mol%									
14:0	1.66 ^{cd}	2.58 ^{a}	1.74 ^{cd}	1.64 ^{cd}	1.52 ^{d}	1.90 ^{d}	2.64 ^{ab}	2.05 ^{cd}	2.19 ^{bc}	1.89 ^{cd}
18:0†	2.87	3.02	3.00	3.05	2.95	2.50	2.77	2.76	2.82	2.71
Gram +										
14:0	0.90 ^{b}	0.72 ^{c}	0.72 ^{c}	0.64 ^{c}	0.63 ^{c}	1.15 ^{a}	1.18 ^{a}	0.97 ^{b}	0.98 ^{b}	0.96 ^{b}
Gram −										
16:1ω9c	1.07	0.98	1.13	0.99	0.95	1.34	1.44	1.33	1.30	1.32
16:1ω7c	4.90	4.68	5.27	4.92	5.01	6.98	6.91	6.99	7.10	7.22
18:1ω5c	2.20^{ab}	2.15^{ab}	2.12^{ab}	2.34^{a}	2.07^{ab}	0.91^{de}	0.44^{d}	0.91^{cd}	0.73^{cd}	1.41^{bc}
17:0cy†	2.60	2.77	2.97	2.95	2.93	2.97	3.15	2.99	2.92	2.96
Fungi										
18:3ω6c	0.81	0.81	0.98	1.07	1.00	0.39	0.14	0.22	0.51	0.13
18:2ω6c	3.53 ^{bc}	5.87 ^{a}	2.42 ^{cd}	2.85 ^{bc}	2.91 ^{bc}	2.00 ^{de}	3.32 ^{b}	1.71 ^{e}	2.04 ^{de}	1.77 ^{de}
Actinomycete										

† No significant 'soil type' effect

Table 3 Relative abundance of bacterial and fungal biomarkers and microbial biomass in the substrate treatments. Data from two soils are combined because there was no soil by treatment interaction. Letters indicate differences among treatment means (n =12). Rows indicate variables that had a significant treatment, but not soil by treatment, interaction (P <0.05)

	Control	Pine	Xylose	Vanillin	Starch
Saturated	Mol %				
16:0	15.15 ^{b}	18.07 ^{a}	15.80 ^{b}	16.32 ^{b}	15.35 ^{b}
Gram +					
i15:0	7.92 ^{a}	6.15 ^{b}	6.60 ^{a}	6.13 ^{b}	6.95 ^{a}
a15:0 †	5.26 ^{a}	4.14 ^{b}	5.01 ^{a}	4.57 ^{b}	4.79 ^{a}
i16:0 †	3.55 ^{a}	2.90 ^{c}	3.39 ^{a}	3.08 ^{b}	3.36 ^{a}
i17:0 †	2.22 ^{a}	1.67 ^{b}	2.05 ^{a}	1.99 ^{a}	2.07 ^{a}
a17:0	2.59 ^{a}	2.03 ^{b}	2.62 ^{a}	2.50 ^{a}	2.60 ^{a}
16:010ME	6.89 ^{a}	5.31 ^{c}	6.29 ^{b}	6.31 ^{a}	6.47 ^{a}
Gram −					
16:1ω5c †	4.30 ^{a}	3.69 ^{b}	4.00 ^{a}	3.87 ^{b}	4.05 ^{a}
18:1ω7c	7.19 ^{a}	8.25 ^{b}	8.42 ^{b}	8.43 ^{b}	7.97 ^{b}
19:0cy	3.34 ^{ab}	2.82 ^{b}	3.70 ^{a}	3.67 ^{a}	3.77 ^{a}
Fungi					
18:1ω9c	6.97^{ab}	7.43^{a}	6.74^{b}	6.92^{ab}	6.82^{b}
Microbial biomass					
pmol PLFA g^{-1}	4975 ^{a}	5995 ^{b}	6060 ^{b}	5745 ^{b}	5855 ^{b}

† indicates no significant 'soil type' effect

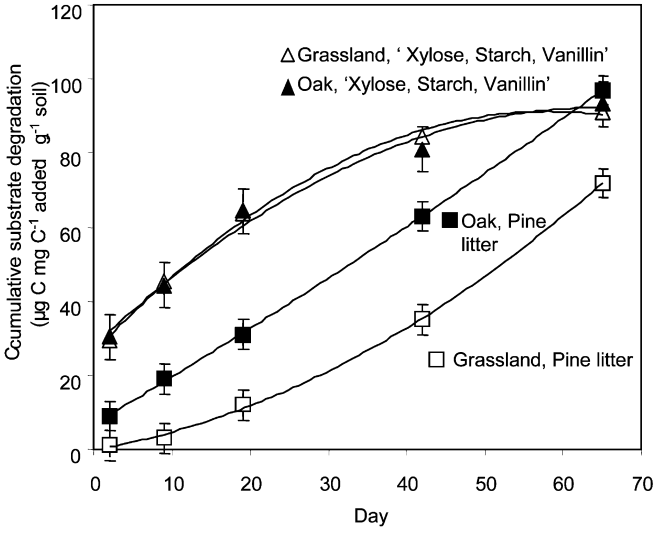

Fig. 2 Cumulative degradation of added substrate in the grassland (*white symbols*) and oak (*black symbols*) soils. *Xylose*, *starch* , *vanillin* is the average of xylose, starch, and vanillin degradation. *Pine litter* indicates averages for pine litter degradation. Substrate degradation rates were normalized to the quantity of substrate added. Values are means ± SE (n =3)

hand, pine litter degradation rates were consistently greater in the oak soil compared to the grassland soil (Fig. 2).

The enhanced degradation of SOM-C following substrate addition (the 'priming effect') was large and long-lived in the grassland soil, but not in the oak soil (Fig. 3). After day 2 there were no significant changes in the cumulative release of soil C in the xylose, vanillin, and starch treatments (Fig. 3). Thus data were pooled into 'xylose, vanillin, starch' and 'pine litter' groups in Fig. 3. Pine litter addition stimulated soil C respiration throughout the 65 day experiment in the grassland soil, and reduced soil C metabolism in the oak soil after day 19 (Fig. 3).

Enzyme activities

Soil enzyme activities tended to increase following substrate additions, but due to high variability, even large increases in enzyme activity were not statistically significant (Table 4). Repeated measures ANOVA revealed that β-glucosidase, galactase, NAGase, and phenol oxidase activities were not significantly increased by substrate treatments. In the grassland soil, only xylosidase activities were significantly increased by a substrate treatment. In oak soils, cellobiohydrolase, α-glucosidase,

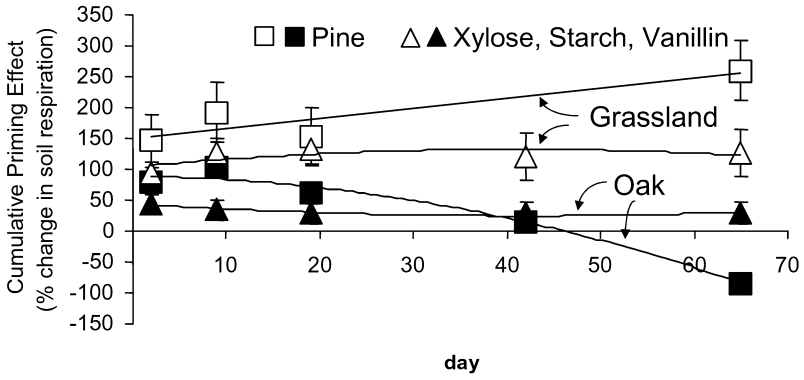

Fig. 3 Cumulative C priming effect (*PE*) following substrate addition in oak and grassland soils. Grassland soils (*white symbols*); oak soils (*black symbols*). Values are means ± SE (n =3)

and xylosidase activities increased in some substrate treatments, β-glucosidase and NAGase tended to increase in the starch and pine treatments. The addition of pine litter or starch caused the most consistent increase in enzyme activities, causing increases in three of the seven enzymes assayed. Starch, although added in much lower concentrations than the pine litter, had a large stimulatory effect on enzyme activities (Table 4). This may have resulted in part from an observed increase in soil aggregation in that treatment. Increased aggregation from starch may have allowed microbial enzymes to be protected from degradation and therefore have a higher enzymatic potential compared to the other substrate treatments (Guggenberger et al. 1999).

We tested whether the enzymatic response (substrate treatment activity over control activity) of the group of enzymes differed between oak and grassland soils using a two-way ANOVA. Principal component 1 of induced enzyme activity values, which incorporated 49% of the enzyme activity data, differed between oak and grassland soils for all substrate treatments (data not shown; F =3.10, P =0.032). The enzymatic response of β-glucosidase, α-glucosidase, and cellobiohydrolase activities were highly related to pine, starch, and vanillin substrate degradation (r^2 range from 0.14 to 0.53, P <0.05, n =24–30), but not xylose or SOM-C degradation.

Incorporation of substrate into biomarkers

Label incorporation into the largest 17 biomarkers (composing 79.0±0.4% of the total microbial biomass) showed several trends. As a group, Gram – biomarkers incorporated more label than the Gram + biomarkers (F =19.78, P <0.0001); typically biomarkers in the oak soil incorporated more substrate than the grassland soil community; and as substrates became more complex (from xylose, starch, and vanillin to pine) the number of differences in biomarker-incorporation values increased between oak and grassland soils (Figs. 4, 5). Typically the oak soil community incorporated more substrate than the grassland soil community early in the experiment (day 9), when the ^{13}C content of PLFAs were initially measured. By day 65, the oak community had incorporated more pine substrate C than the grassland community. Principal components analysis of the ^{13}C-PLFA data for substrate incorporation was used to calculate an aggregated 'functional group' that degrades each of the four added substrates. ANOVA of the functional group principal component data showed that the groups degrading labile substrates (xylose, starch, and vanillin) do not differ by plant community type while the functional groups that

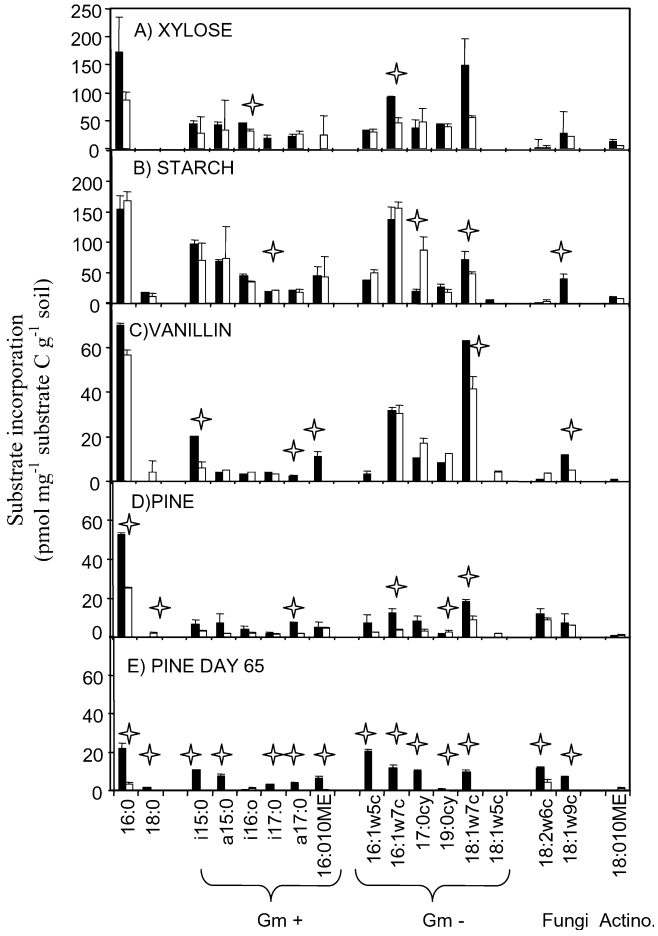

Fig. 4 Incorporation of four different substrates into PLFA biomarkers by oak (*black bars*) and grassland communities (*white bars*). *Panels A–D* represent 9 days of incubation and *panel E* represents 65 days of incubation. Substrate incorporation was normalized to the amount of substrate added. Data are mean ± SD (n =3). *Asterisks* indicates significant differences (P <0.05) in substrate incorporation between oak and grassland soils

Table 4 Average enzyme activities of oak and grassland soils following substrate additions. Values are means ± SE averaged across all time points (n =15, P <0.05). 'Effect' represents the significant effect in the repeated measures ANOVA. Bold values indicate differences from control. Letters denote differences among treatment means for each column. Values in bold in xylosidase column indicate significant treatment effect, but there was no soil by treatment interaction

	Cellulases				Hemicellulases		Ligninase
	β-glucosidase (mol $h^{-1}g^{-1}$)	Cellobiohydrolase (mol $h^{-1}g^{-1}$)	Galactase (mol $h^{-1}g^{-1}$)	α-glucosidase (mol $h^{-1}g^{-1}$)	Xylosidase (mol $h^{-1}g^{-1}$)	NAGase (mol $h^{-1}g^{-1}$)	Phenol oxidase (mg $h^{-1}g^{-1}$)
Significant effect	Soil	Soil × Substrate	Soil	Soil × Substrate	Soil and Substrate	NS	Soil
Grassland							
Control	1.24±0.13	0.35±0.07 ^{c}	0.27±0.05	0.43±0.08 ^{c}	0.37±0.09	1.03±0.19	5.1±0.7
Pine	1.36±0.17	0.50±0.05 ^{c}	0.37±0.06	0.42±0.06 ^{c}	**0.76±0.11**	1.32±0.18	6.5±0.8
Starch	1.43±0.21	0.41±0.05 ^{c}	0.20±0.03	0.36±0.04 ^{c}	**0.48±0.08**	1.94±0.92	8.3±1.0
Vanillin	1.32±0.12	0.61±0.08 ^{c}	0.23±0.05	0.37±0.06 ^{c}	**0.68±0.22**	1.42±0.11	6.9±0.8
Xylose	1.76±0.39	0.62±0.09 ^{c}	0.27±0.05	0.46±0.05 ^{c}	0.44±0.05	1.46±0.19	6.3±0.8
Oak							
Control	3.63±0.63	0.90±0.09 ^{b}	0.32±0.05	0.62±0.05 ^{b}	0.82±0.15	1.91±0.30	9.0±1.0
Pine	4.41±0.54	**1.13±0.11** ^{a}	0.38±0.05	**0.85±0.04** ^{a}	**1.18±0.13**	2.46±0.30	9.3±1.2
Starch	4.39±0.64	**1.56±0.18** ^{a}	0.39±0.05	**1.18±0.08** ^{a}	**1.38±0.16**	3.12±0.36	8.2±1.2
Vanillin	3.94±0.59	0.96±0.70 ^{b}	0.51±0.07	0.77±0.07 ^{b}	**1.07±0.13**	1.73±0.27	8.7±1.1
Xylose	3.68±0.60	1.01±0.85 ^{b}	0.33±0.05	0.71±0.05 ^{b}	0.84±0.11	1.83±0.29	8.9±0.9

Fig. 5 Principal component representation of substrate incorporation into PLFA biomarkers in grassland (*white symbols*) and oak soils (*black symbols*). Only the pine degrading functional group differed by plant community type. Values are means ± SE (n =3)

degrade pine litter do differ by plant community type (Fig. 5).

Incorporation of SOM-C into biomarkers

SOM-C incorporation in Gram – biomarkers was greater than Gram + biomarkers as a group, regardless of soil or substrate, suggesting, as with simple C compounds, that Gram − organisms are very active heterotrophs (Fig. 6). In the starch, xylose, and vanillin substrate treatments, SOM-C was incorporated typically into the most abundant bacterial biomarkers. Most biomarkers within the oak soil community tended to incorporate more SOM-C than biomarkers in the grassland soil when xylose, vanillin, or starch was added, although only a subset were significant (Fig. 6A–C). In the pine litter treatment, only two oak soil biomarkers (18:0 and 18:1ω5c) incorporated soil C, while seven grassland soil biomarkers incorporated SOM-C. An ANOVA of the first two principal components generated from the ^{13}C-PLFA data resulted in significant soil by treatment interactions (F =12.1; P =0.003 for PC1 and F =11.6, P =0.0003 for PC2) revealing that the functional groups degrading SOM-C are specific to the particular plant community type (Fig. 7).

Because pine litter was being actively degraded on day 65 and there were large differences in the priming effect, we specifically compared ^{13}C-PLFA data between grassland and oak soils for this date. Soil organisms in the grassland soil were primarily utilizing SOM-C (Fig. 6E) while the oak soil community was primarily degrading pine substrate (Fig. 4E), consistent with measured process rates of SOM-C (Fig. 3) and pine litter respiration (Fig. 2).

Discussion

Distinct microbial communities and C-degrading functional groups were associated with oak and grassland plant communities in soils that were climatically and edaphically similar, indicating the tight coupling of microbial communities with plant communities, even for the largely saprophytic microbial community (Fig. 1). Different plant

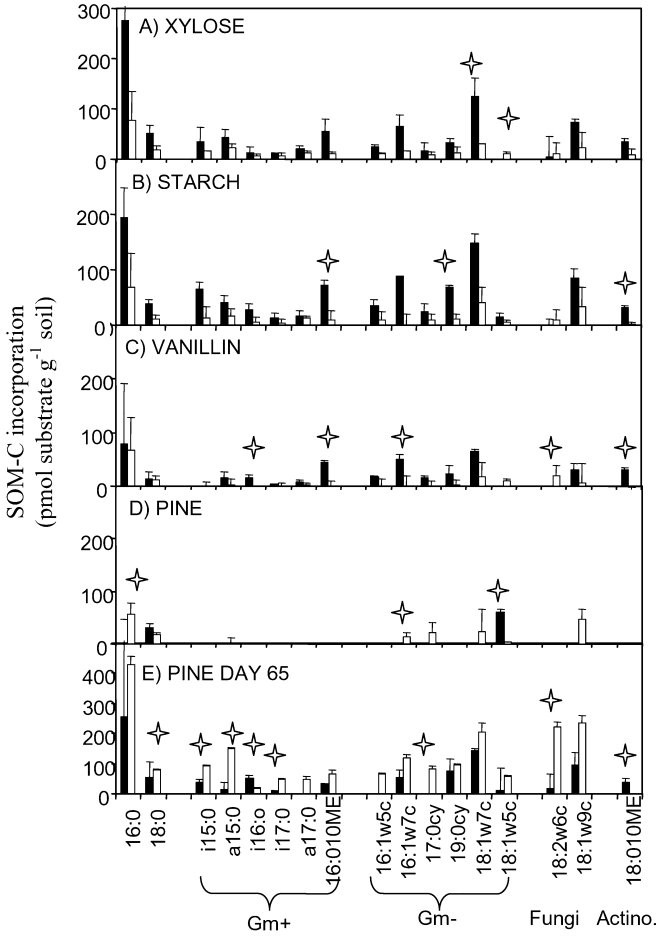

Fig. 6 SOM-C incorporation into PLFA biomarkers of grassland (*white bars*) and oak (*black bars*) microbial communities following additions of four different substrates. *Panels A–D* represent 9 days of addition and *panel E* represents 65 days of incubation. SOM-C incorporation was normalized to the amount of substrate added.

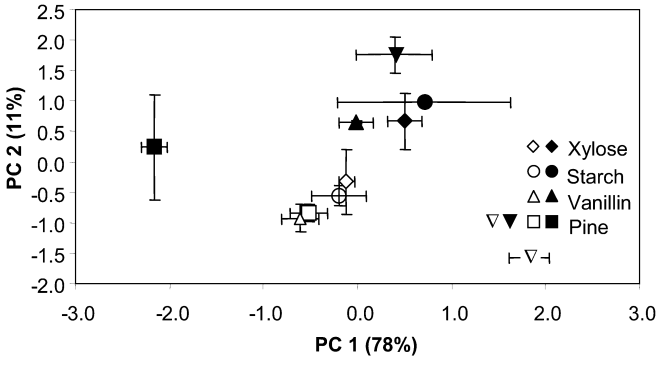

Fig. 7 Principal component representation of SOM-C degrading functional groups following substrate additions in grassland (*white symbols*), and oak (*black symbols*) soils at day 9 for all substrates and a second sampling at day 65 for pine (*downward triangle*). The SOM-C degrading functional group differed by soil type. Values are means ± SE (n =3)

species can be associated with different microbial communities as evidenced by fatty acid (Myers et al. 2001), physiological (Myers et al. 2001; Westover et al. 1997), and DNA techniques (Kuske et al. 2002). Substrates provided by plant litter are an important controller of microbial community composition, as evidenced by the changes in microbial community composition that occurred following pine and vanillin addition (Fig. 1).

The ^{13}C-PLFA technique defined microbial biomarkers that were indicative of organisms that degrade different C substrates. Patterns of label incorporation into biomarkers were generally consistent with the observed ^{12}C-PLFA results, which showed that Gram – biomarkers had a greater positive response to the added substrates compared to Gram + organisms. Increases in monounsaturated Gram – biomarkers and decreases in branched Gram + biomarkers have been observed following substrate additions in several studies (Bossio et al. 1995; Phillips et al. 2002). Because all substrates were incorporated into nearly all biomarkers, it was not possible to identify simple groups (potentially 'functional groups') responsible for specific C substrate utilization based upon one or a few fatty acid biomarkers. Using Principal Components Analysis however, it was possible to construct a 'fingerprint' of the different functional groups and determine if they differed by plant community type (Figs. 5, 7).

We determined that simple substrates were degraded by the same groups of organisms in both soils, and at similar rates, but pine litter was degraded by different microbial groups in the two soils, and at different rates. Thus as substrate complexity increased, the functional group responsible for its degradation became more distinct between the two soils. This is consistent with the hypothesis that the functional groups that degrade macromolecular or recalcitrant C are not as diverse or functionally redundant across ecosystems (Schimel and Gulledge 1998). Functional groups that degraded simple substrates were indistinguishable between oak and grassland soils, even though overall community composition differed by plant community type (Fig. 5). These results may help to explain why in some experiments a change in composition is not related to a change in function; when analyzing whole microbial community composition, shifts in composition may occur that are unrelated to the process being measured.

The enzymatic response to substrate additions was greater in the oak soil compared to the grassland soil and the enzymatic responses often correlated with substrate degradation, suggesting that enzyme activities are highly related to substrate degradation patterns. The significant relationship between the degradation of individual C compounds and soil enzyme activities suggests that the enzyme activities are a limiting step in decomposition. However, the degradation rates of simpler C compounds did not differ between the two soils, suggesting that the degradation rates of simple compounds are unaffected by differences in microbial community composition and associated enzymatic potential. The lower enzymatic response of the grassland microbial community following substrate addition (Table 4) may partially explain its lowered capacity for pine litter degradation as measured by lower pine litter respiration rates (Fig. 2) and reduced

incorporation of pine litter carbon into fatty acid biomarkers (Fig. 4).

The lower specific microbial respiration (Table 1), lower relative abundance of fungi (Table 2), and lower incorporation of SOM-C into microbial biomarkers (Fig. 6) in the oak soil suggests that SOM quality in this soil is lower than the grassland soil and not as available for microbial metabolism. Oak litter and oak soil organic matter can be high in condensed tannins (Deschaseaux and Ponge 2001; Lopez Llorca and Olivares Bernabeu 1997), which can complex with cell-free proteins and may result in lower SOM-C metabolism by fungi and other organisms (Harrison 1971). This effect was most apparent on day 65, when the microbial communities in the two soils were primarily utilizing and respiring different sources of C in the pine litter treatment; the oak community was primarily degrading pine litter C (Fig. 4), and the grassland community was primarily degrading SOM-C (Fig. 6). Pine litter in the oak soil appears to have remained free of an inhibitory affect of tannins and provided an available source of C for microbial degradation.

In this study, priming effects lasted several months, substantially increasing SOM-C respiration in the grassland soil and decreasing SOM-C respiration in the oak soil (Fig. 3). The priming effect is an important part of C cycling research because the magnitude of the effect can be quite large in field soils (Kuzyakov et al. 2000), given that plants release C into the soil for much of the year. It is significant that the same substrate can cause different PE responses (even positive or negative) in different soil types (Fig. 3). This type of response emphasizes the fact that the controls on PE are not well understood. Models of C cycling typically consider SOM as pools of C with individual decomposition and transfer rates. The interaction of one pool with another (e.g. the priming effect) is not explicitly considered, although the priming effect may be indirectly included in decomposition rates of soil C pools. In this study, ^{13}C-PLFA provided some resolution of the microbial groups responsible for the priming effect, showing that the functional groups accessing the SOM-C pool differed between the grassland and oak soils, in part through differences in fungal activity (Figs. 6, 7).

The interactions between plant and microbial communities can alter carbon cycling in terrestrial ecosystems by altering the quantity and quality of available substrates, as well as the composition of the microbial community and its enzymatic capacity for substrate degradation. We found different microbial communities associated with different plant communities, yet very similar groups of soil microorganisms utilized simple carbon substrates (starch, xylose, vanillin) while the microbial groups utilizing more complex or recalcitrant compounds (pine litter and SOM) differed significantly between the plant communities. As plant communities are impacted by events such as land use change or plant invasions, the feedback to C and N cycling will in part be dependent on changes in the associated microbial communities. As yet, we are not well positioned to predict how microbial communities should be expected to change and how these changes might impact ecosystem processes. Our results are a step in this direction, demonstrating that soil microbial groups responsible for carbon degradation can be defined using ^{13}C-PLFA and hence the mediation by soil microorganisms of plant community impacts on decomposition processes can now be identified and quantified.

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