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Carbon flow from ^{13}C-labeled straw and root residues into the phospholipid fatty acids of a soil microbial community under field conditions

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Abstract

To better understand how residue quality and seasonal conditions influence the flow of C from both root and straw residues into the soil microbial community, we followed the incorporation of ^{13}C-labeled crimson clover (*Trifolium incarnatum*) and ryegrass (*Lolium multiflorum*) root and straw residues into the phospholipid fatty acids (PLFA) of soil microbial biomass. After residue incorporation under field conditions in late summer (September), the ^{13}C content of soil PLFA was measured in September, October, and November, 2002, and April and June, 2003. Multivariate non-metric multidimensional scaling techniques showed that the distribution of ^{13}C among microbial PLFA differed among the four primary treatments (ryegrass straw and roots, clover straw and roots). Regardless of treatment, some PLFA remained poorly labeled with ^{13}C throughout much of the study (16:1ω5, 10Me17:0; 0–5%), whereas other PLFA consistently contained a larger percentage of residue-derived C (16:0; 18:1ω9, 18:2ω6,9; 10–25%). The distribution of residue ^{13}C among individual PLFA differed from the relative contributions of individual PLFA (mol%) to total PLFA-C, suggesting that a subset of the soil biomass was primarily responsible for assimilating residue-derived C. The distribution of ^{13}C among soil PLFA differed between the sampling times, indicating that residue properties and soil conditions influenced which members of the community were assimilating residue-derived C. Our findings will provide the foundation for further studies to identify the nature of the community members responsible for residue decomposition at different times of the year, and what factors account for the dynamics of the community involved.

Keywords: Residue decomposition; ^{13}C-labeled plant residues; ^{13}C-PLFA compound specific isotope analysis of microbial communities; C flow from plant residues into soil microorganisms

1. Introduction

Microorganisms are primary regulators of nutrient cycling and the conduit through which plant root and shoot residues are decomposed. Although it is well established that root and shoot residues can decompose at different rates, and persist in microbial and soil C pools for time periods that vary considerably (Berg et al., 1987; Muller et al., 1988; Gale et al., 2000; Puget and Drinkwater, 2001; Lu et al., 2003; Loya et al., 2004), the corresponding responses and dynamics of the microbial community are only vaguely understood and poorly quantified. Studies that follow the seasonal dynamics of C movement from different residue components into microbial communities may help to identify the types of microbes that are involved in residue decomposition at different times of the year, and provide more insights into the interactions between residue components and environmental conditions on soil microbial community dynamics. Recently, reports have appeared in the literature describing attempts to follow the changes in microbial community composition accompanying plant residue decomposition (Thirup et al., 2001; Nakamura et al., 2003; Aneja et al., 2004; Malosso et al., 2004; McMahon et al., 2005). In several of these studies, the method used to examine community composition involved extracting and quantifying the fatty acids associated with

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the phospholipid fraction of soil microbial biomass. Phospholipid fatty acids (PLFA) are essential membrane components of living cells, and because phospholipids rapidly degrade following cell death, they are excellent biomarkers of viable microorganisms. Furthermore, some PLFA are useful biomarkers of specific microbial groups, and provide information on the dynamics of microbial communities (White, 1993; White and Ringelberg, 1998). Several studies have successfully traced the flow of C from ^{13}C-labeled simple substrates into the PLFA fraction of native microbial communities (Abraham et al., 1998; Bossio et al., 1998; Arao, 1999; Boschker et al., 1998; Hanson et al., 1999; Phillips et al., 2002; DeForest et al., 2004). In addition, recent laboratory-based studies have used ^{13}C to trace the movement of complex plant-derived C from root exudates (Butler et al., 2003; 2004; Lu et al., 2004), leaves (Malosso et al., 2004), pine needles (Waldrop and Firestone, 2004), and ryegrass straw residues (McMahon et al., 2005) into PLFA of soil microbial communities.

The purpose of our study was to trace the movement of ^{13}C from field-labeled straw and root residues into the microbial PLFA under field conditions, and to determine if C from straw and root residues of both a field-grown legume and a grass with different C:N ratios might direct the flow of C into different members of the soil microbial community. We also wanted to determine if the incorporation of C into the microbial community changed over a period of time under diverse western Oregon field conditions that ranged from transient rewetting/drying of air-dry and warm soil in the fall (September–November), through a sustained water-saturated and cold (non-freezing) soil environment during the winter (November–April), and into the spring growing season (April–June) when soil temperature and soil water content are optimum for microbial activity.

2. Materials and methods

2.1. Site description

The field plots were located on a Woodburn silty clay loam (Aquultic Argixerolls) located at the Hyslop Agricultural Field Laboratory, Oregon State University, Corvallis. The soil at the field site has a long history of being conventionally tilled and cropped to rotations that include small grains, forage legumes, and grasses. The experimental design was a randomized complete block with four replicates per treatment. Each 30 by 5-m replicated block was seeded with either annual ryegrass (*Lolium multiflorum* Lam.) or crimson clover (*Trifolium incarnatum* L.). Plots were seeded in September 2001, and germinated in response to fall rain. The herbicide, clethodim, was applied to clover plots in late February to control annual blue grass.

2.2. ^{13}C enrichment of plant biomass

Within each treatment block, ^{13}C enrichment of plant shoots and roots was accomplished by repeated pulse labeling of each plant species between the months February to June. This labeling was accomplished using a 320-l volume ($0.6\times0.6\times0.75$ m; length by width by height) plexiglass chamber that was placed over the sub-plots and sealed at the soil surface. $^{13}CO_2$ was generated by adding HCl to portions of 99% ^{13}C-enriched $NaHCO_3$ (Cambridge Isotope Labs., Andover, MA) and $^{13}CO_2$ was injected into the plexiglass chamber via an injection port. The concentration of CO_2 in the chamber was raised by approximately 400 μmol mol^{-1} air. After the CO_2 in the chamber dropped to 150 μmol mol^{-1} of air, more $^{13}CO_2$ was injected. This process was repeated three times until a total of 600 mg of ^{13}C had been added to each chamber. Subsequently, an identical quantity of tank CO_2 (99% CO_2; δ ^{13}C-PDB $= -36.5$‰) was injected into the chamber to ensure that most of the $^{13}CO_2$ respired by the plants was re-incorporated. Plants were pulse-labeled 5 or 6 different times during the growing season, commencing in mid-February and ending in May 2002. Plants were harvested during July after seed maturation, and their yields and ^{13}C contents assessed. Biomass yields of ryegrass roots and shoots were 210 and 920 g m^{-2}, respectively. Standing biomass of clover roots and shoots was 200 and 800 g m^{-2}, respectively. The C:N ratios of clover and ryegrass roots were 36 (%N = 1.25) and 75 (%N = 0.60), respectively, whereas clover and ryegrass straw were 44 (%N = 1.0) and 125 (%N = 0.36), respectively. At maturity, the mean δ ^{13}C-PDB values of ryegrass and clover straw were +91 and +108‰, respectively. ^{13}C contents of ryegrass and clover roots were enriched, yet were not significantly different from each other ($\delta^{13}C = +31$‰).

2.3. Experimental design: incorporation and incubation of ^{13}C-labeled residues

After harvest of ^{13}C-labeled straw, soil was excavated from 0 to 20-cm depth of each labeled subplot, and also from equal size subplots of unlabeled plants. Appropriate amounts of straw material equivalent to ~950 g m^{-2} were thoroughly mixed with the excavated soil samples so that they contained both roots and shoots of one plant species. In a plot of land immediately adjacent to the field site, four randomly assigned blocks were seeded with crimson clover and annual ryegrass. In each of the four blocks of each species, three subplots (0.6×0.6 m) were excavated to receive the following treatments: (1) excavated soil containing root-derived C/root residue and straw residue of ambient δ^{13}C; (2) ^{13}C-enriched straw and excavated soil containing unlabeled root-derived C; (3) excavated soil containing ^{13}C- enriched root derived C/root residue and unlabeled straw. Soil plus residue treatments were mixed into the plots on September 1 2002. At time intervals after

residue incorporation, (September, October, November, 2002, April, and June, 2003), soil and residues from each treatment were sampled (0–18-cm depth) with a 5-cm diameter PVC core at three randomly chosen points within each plot. Samples were homogenized, residues removed manually, and soil sieved to pass a 5-mm mesh.

2.4. Extraction and analysis of soil PLFA

Soil phospholipid fatty acids (PLFA) were extracted and analyzed after removal of straw and root residues. Lipids were extracted according to the procedure of White and Ringleberg (1998) as modified by Butler et al. (2003). Briefly, portions of soil (15 g wet weight) were extracted overnight in a mixture of chloroform, methanol, and 50 mm phosphate buffer (pH = 7.1). Lipids were extracted the following day by centrifugation and filtration. The phospholipid fraction was recovered and saponified to obtain fatty acid methyl esters (FAME), and then analyzed by capillary GC–combustion–isotope ratio mass spectrometry (GC–C–IRMS). Chromatographic peaks were quantified using 13:0 and 19:0 methyl ester standards.

The $\delta^{13}C$ values of individual PLFA were determined as described by Butler et al. (2003) with an Agilent 6890 gas chromatograph (Agilent, Inc., Palo Alto, CA) equipped with a 30-m HP Innowax column (internal diameter, 0.25 mm; film thickness, 0.25 μm) connected to a Europa ORCHID on-line combustion interface in line with a Europa 20–20 mass spectrometer (Europa Scientific, Cheshire, England). During the methylation step an additional C atom is added to the fatty acid molecule. This additional C atom, of known $\delta^{13}C$ value (−45‰), was corrected for the $\delta^{13}C$ values of the PLFA with the following equation

$$\delta^{13}C = [(C_{PLFA} + 1)\delta^{13}C - \delta^{13}C]/C_{P-FAME} \quad (1)$$

where C_{PLFA} and $\delta^{13}C_{PLFA}$ refer to the number of C atoms and the $\delta^{13}C$ value, respectively, of the PLFA; $C_{P\text{-}FAME}$ and $\delta^{13}C_{PFAME}$ refer to the number of C atoms and the $\delta^{13}C$ value of the fatty acid methyl ester after derivitization, and $\delta^{13}C_{MeOH}$ refers to the $\delta^{13}C$ value of the methanol used for methylation.

We calculated the proportion of residue derived PLFA-C for each individual PLFA by using the following equation

$$F_i = M_c \frac{(\delta_{li} - \delta_{ui})}{(\delta_{gi} - \delta_{ui})}; \quad i = \text{individual PLFA} \quad (2)$$

where F_i is the relative fraction of C in each PLFA composed of enriched ^{13}C residue, M_c is the relative percentage of C in each PLFA to that of the total PLFA, δ_l represents the $\delta^{13}C$ of the PLFA-C derived from the ^{13}C enriched residue, δ_u represents the $\delta^{13}C$ of the PLFA-C derived from un-enriched residue, and δ_g represents the $\delta^{13}C$ of the labeled residue.

The proportion (P_{Fi}) of residue derived ^{13}C to that of the total residue-derived PLFA was then calculated for each fatty acid at each sampling time, and for each residue-type and replication using the following equation

$$P_{F_i} = \frac{F_i}{\sum F_i} \times 100 \quad (3)$$

Standard nomenclature is used to describe PLFA. The number before the colon refers to the total number of C atoms; the number(s) following the colon refers to the number of double bonds and their location (after the 'ω') in the fatty acid molecule. Notations: 'Me,' 'cy,' 'i,' and 'a' refer to methyl group, cyclopropane groups, and iso- and anteiso-branched fatty acids, respectively. Thirteen PLFA comprised 84–90% of the PLFA, and were each present in sufficient quantity to obtain accurate $\delta^{13}C$ values. Other indicator fatty acids such as hydroxy fatty acids and long chain fatty acids (≥C20) were either below the limit of accurate detection, or present in amounts too small to get accurate $\delta^{13}C$ values. Two fatty acids (16:1ω7 and 10Me16:0) merged (denoted as 16:1+) into one peak, as did 18:1ω7 and 10Me18:0 (denoted as 18:1+).

2.5. Statistical analyses

Both treatment and sampling time effects on mol% and as a proportion residue derived C in each PLFA (PF_i) from roots and straw were assessed using a repeated measures ANOVA analysis (SAS, 1996). Where appropriate, LSD comparisons were calculated and considered significant at $p < 0.05$ for mol% PLFA, and $p < 0.01$ for P_{Fi}. Class statements were treatment (clover roots, clover shoots, ryegrass roots, and ryegrass shoots) and replication; replication (block) was analyzed as the random effect and time as the repeated measure. Though straw and roots were mixed together and homogenized in early September, root C was composed of a mixture of both root residues and root-derived C that had been deposited into the soil during the growing season and during the summer dormant period. Comparisons between root and straw derived PLFA-C were made with these differences in mind.

Residue type and sampling time were analyzed conjointly and separately on mol% PLFA and PFi using a multivariate method: Multi-response Permutation Procedure (MRPP). The MRPP is a nonparametric procedure for testing the hypothesis of no difference between two or more groups of entities (McCune and Grace, 2002). This method possesses the advantage of not requiring multivariate normality nor homogeneity of variances. Non-metric Multidimensional Scaling (NMS), also a non-parametric method was used to provide graphical ordination of the experimental data displayed with two synthetic axes. Data were transformed by treatment using the 'general relativization' procedure in PC-ORD. 'Relativization' in multivariate analysis removes the potentially strong influence of absolute abundance on community data. 'Indicator species' analysis and correlations with the main matrix are presented when

appropriate to facilitate the identification of variables that are important descriptors of treatment and time differences (McCune and Grace, 2002). To clearly express the relationships in the NMS plots, we averaged the five sampling times of mol% PLFA for both the clover and ryegrass treatments. The standard error associated with the five different sample times was relatively small, as indicated in the NMS plots.

3. Results

3.1. Temporal dynamics of straw and root derived C within the microbial community

Data presented in Fig. 1 show the mol% distribution of 13 analytically distinguishable PLFA extracted from the September samples of soil and root residues. Although the overall mol% distributions of PLFA among the ryegrass and clover treatments were similar (Fig. 1b), the contributions of root-derived C differed among the individual PLFA of each of the two treatments (Fig. 1a). For example, in the ryegrass treatment root-derived C contributed between 15–20% (16:0, 18:1+) and 0% (18:0, cy19:0) of PLFA-C. In the clover treatment, root-derived C contributed between 10 and 15% (16:0, 18:1+, 18:2ω6,9), and 0% (10me17:0, cy19:0). Furthermore, significant treatment differences were measured for the contributions of root-derived C in six of 13 PLFA. At the time of straw incorporation, root-derived C comprised between 29% (clover) and 38% (ryegrass) of total PLFA-C (Fig. 2b and c). After a large decline between October and November, the contribution of ryegrass root-derived C to PLFA-C stabilized for the remainder of the incubation (~25% of PLFA-C in June). In contrast, clover root-derived PLFA-C declined steadily between September and November, and sharply between April and June and ultimately accounted for ~10% of total PLFA-C (Fig. 2b

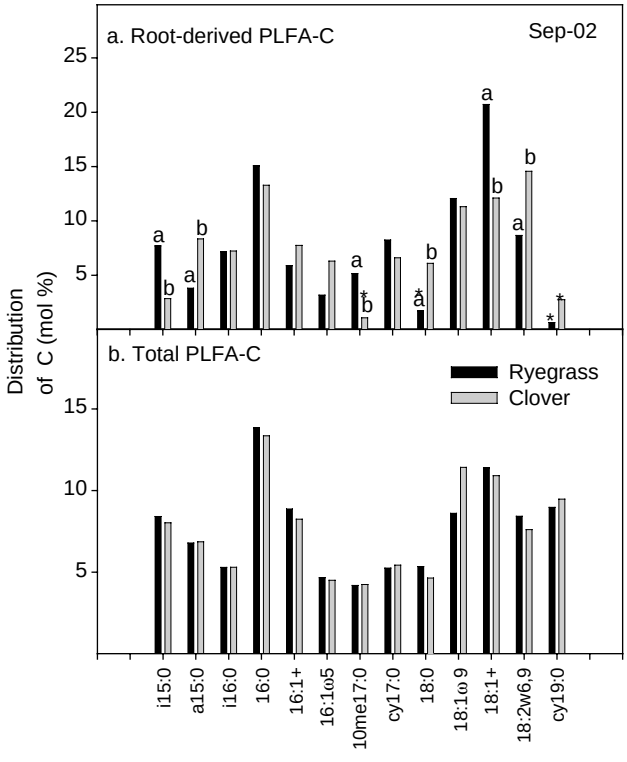

Fig. 1. The proportional (P_{Fi}) distribution of PLFA-C from root-derived C (a) and distribution of total (mol%) PLFA-C (b) in soil sampled September, 2002. Absence of vertical bars or designation by an asterisk denotes when a specific PLFA is not considered significantly different from zero. Small case letters designate significant differences between clover and ryegrass treatments for that specific PLFA and sampling date ($n=4$; $p<0.01$).

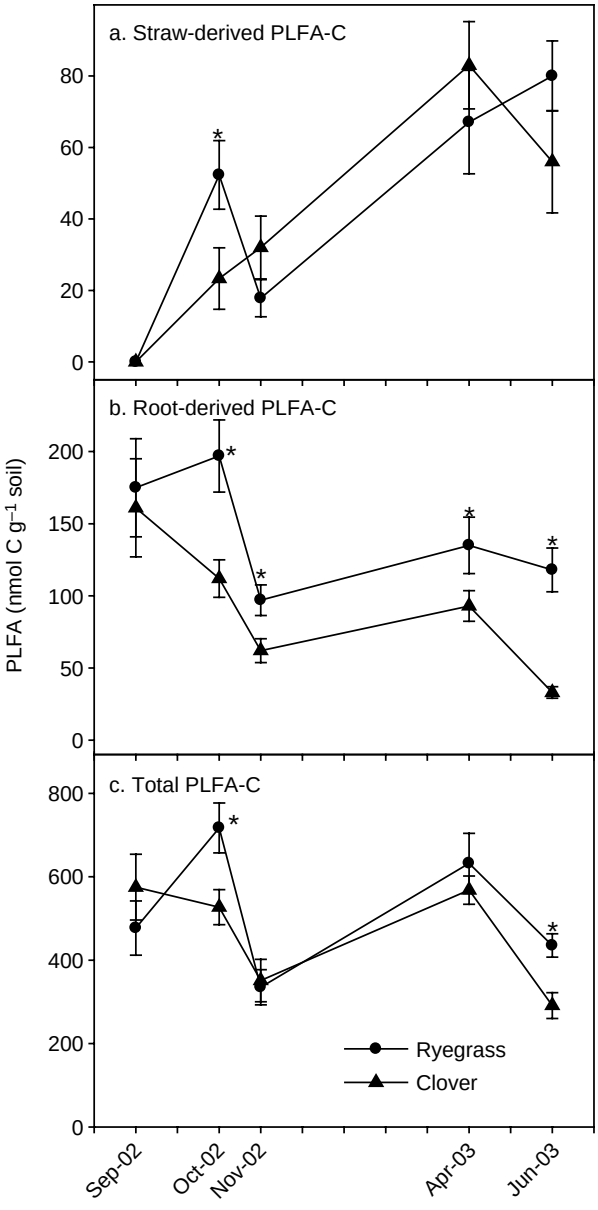

Fig. 2. Nanomoles of PLFA-C derived from (a) straw-C (b) root-C and (c) the total nmol PLFA-C g^{-1} soil. Symbols and bars are indicative of the mean and standard error ($n=4$) associated with each sampling date. Asterisks denote significant differences between clover and ryegrass treatments ($n=4$; $p<0.05$).

and c). Throughout the study, the amount of PLFA-C derived from ryegrass root C was always greater than the contribution from ryegrass straw C (Fig. 2a and b). In contrast, the contribution of clover root-derived C had declined to equal, or less than the contribution from straw in both April and June (Fig. 2a and b).

3.2. Plant species effects on straw-C incorporation into the microbial community

Although incorporation of residues resulted in an immediate flux of straw-derived C into soil PLFA between September and October (Fig. 2a), straw-derived C only represented a small portion (4–7%) of the total soil pool of PLFA-C. These percentage values generally increased over the winter period (November–April), yet, they never contributed more than 20% (ryegrass, June) and 15% (clover, April) of the total soil PLFA-C (Fig. 2a,c). An NMS analysis revealed that the percent distributions of PLFA-C derived from both clover and ryegrass straws were significantly different from the distribution of total PLFA-C on three occasions (October, $p<0.01$, Figs. 3 and 4; November and June, $p<0.05$, Figs. 3, 5 and 7). Several

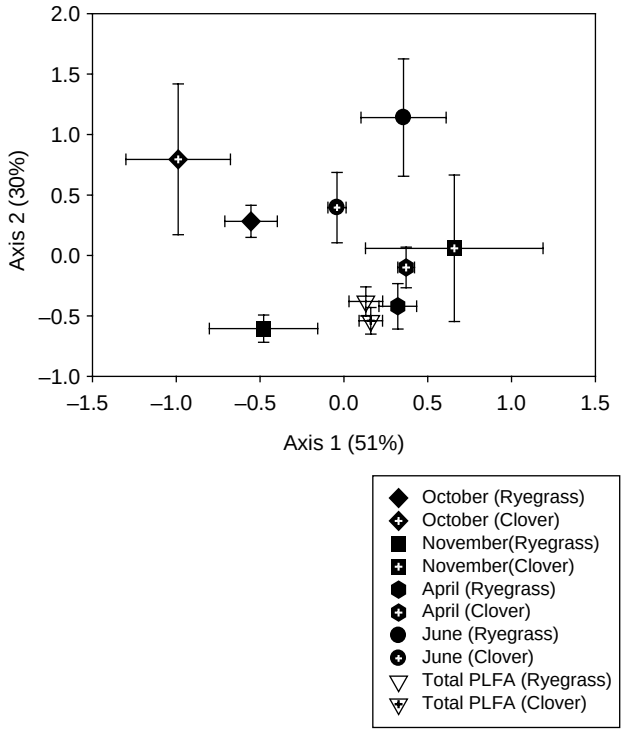

Fig. 3. The multivariate analysis of the proportional (P_{Fi}) distribution of straw derived ^{13}C and total straw-derived C in PLFA. The first and second axis using non-metric multidimensional scaling (NMS) are shown. The variability associated with mol % PLFA-C through the annual cycle was small compared to distribution of residue-derived PLFA-C. Hence, seasonal treatment means of mol% PLFA-C for clover and ryegrass were averaged for the purpose of NMS and multivariate statistical analysis. Percentages denote the amount of variability associated with each axis. Values of symbols and bars represent means and standard error, respectively (n=4).

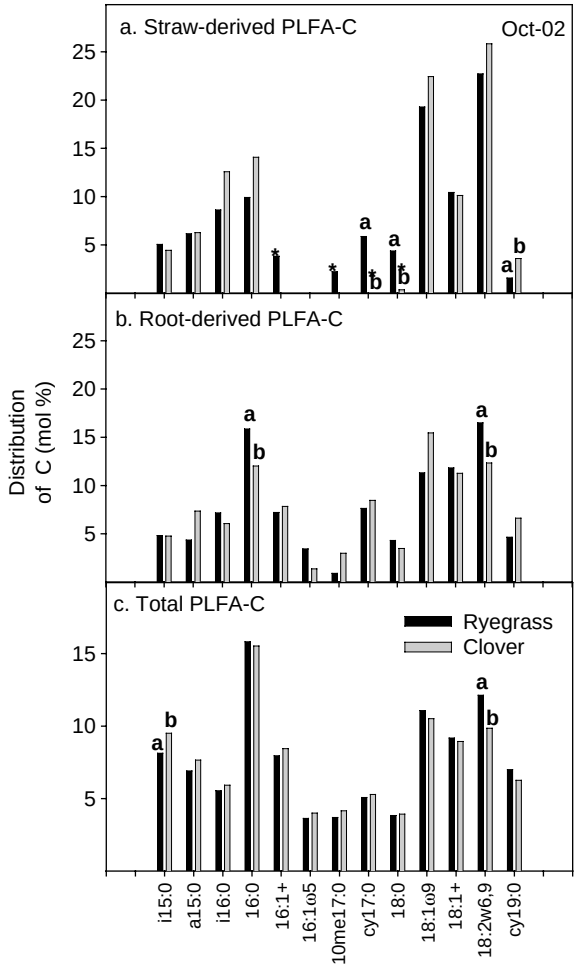

Fig. 4. The proportional (P_{Fi}) distribution of PLFA-C derived from straw (a), root-derived C (b) and the distribution of total (mol%) PLFA-C (c) in soil sampled October, 2002. Absence of vertical bars or designation by an asterisk denotes when a specific PLFA is not considered significantly different from zero. Small case letters designate significant differences between clover and ryegrass treatments for that specific PLFA and sampling date ($n=4$; $p<0.01$).

PLFA (i16:0, 16:0, 16:1+, cy17:0, 18:1+, cy19:0) were strong 'indicator species' ($p<0.01$) of temporal changes in the flow of straw C to the PLFA of the soil microbial community. However, no individual PLFA was a strong indicator species that consistently described the differences between ryegrass and clover.

Overall, the distributions of clover and ryegrass straw C among PLFA were significantly different from each other in November and June ($p<0.05$, Figs. 3, 5 and 7), but not in October and April (Figs. 3, 4 and 6). The contribution of straw-derived C to the C content of some soil PLFA amounted to >20% in October (18:1ω9 and 18:2ω6,9), whereas there was no significant incorporation of either ryegrass or clover straw C into other PLFA (16:1+, 16:1ω5, and 10Me17:0) of the same sample (Fig. 4). In addition, no significant amount of clover straw-derived C was found in either cy17:0 or 18:0. In November, however, significant amounts of straw-C were found in all of

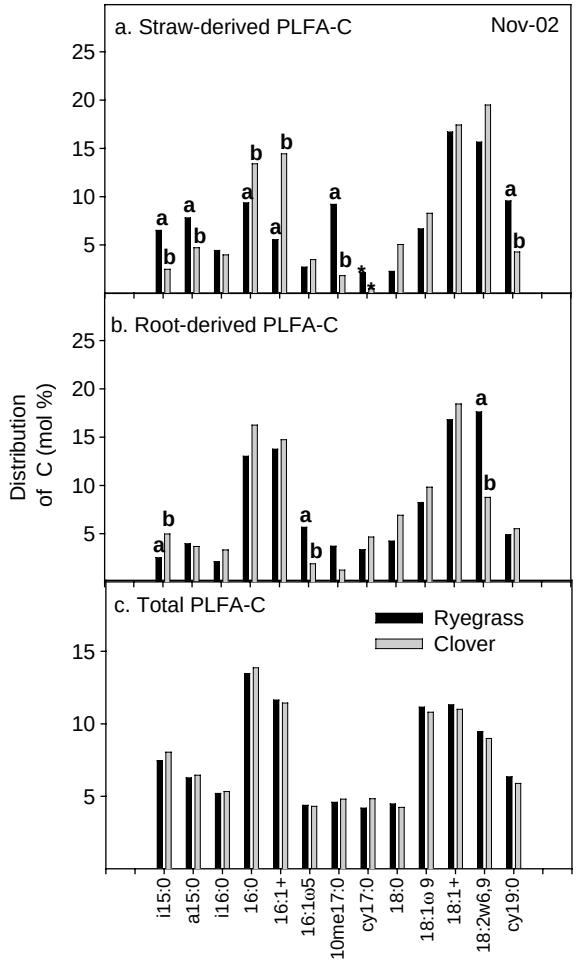

Fig. 5. The proportional (P_{Fi}) distribution of PLFA-C from straw (a), root-derived C (b) and the distribution of total (mol%) PLFA-C (c) in soil sampled November, 2002. Absence of vertical bars or designation by an asterisk denotes when a specific PLFA is not considered significantly different from zero. Small case letters designate significant differences between clover and ryegrass for that specific PLFA and sampling date ($n=4$; $p<0.01$).

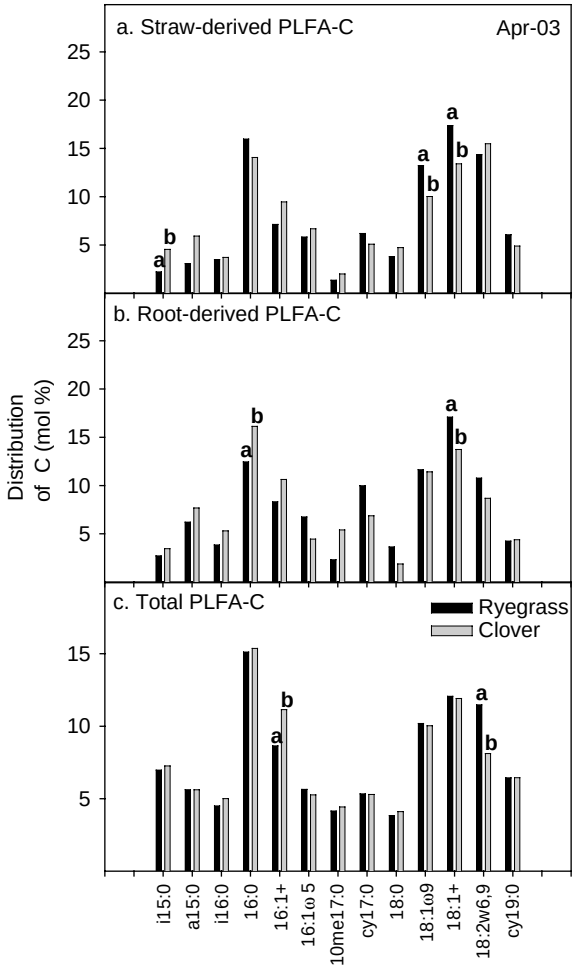

Fig. 6. The proportional (P_{Fi}) distribution of PLFA-C from straw (a), root-derived C (b) and the distribution of total (mol%) PLFA-C (c) in soil sampled April, 2003. Absence of vertical bars or designation by an asterisk denotes when a specific PLFA is not considered significantly different from zero. Small case letters designate significant differences between clover and ryegrass for that specific PLFA and sampling date ($n=4$; $p<0.01$).

the above mentioned PLFAs except for cy17:0 (Fig. 5). Furthermore, during the October–November time period, there were significant increases of ryegrass straw-derived C into cy19:0, and of both types of straw-C into 18:1+. In contrast, there was a large decline in the proportion of straw-derived C in i16:0, 18:1ω9 and 18:2ω9. No significant changes occurred in the distribution of clover straw C among PLFA-C between November and April, whereas, in the case of ryegrass straw, significant changes occurred in distribution over this time period (Fig. 3). For example, significant reductions occurred in the % of ryegrass straw C in a15:0, 10Me17:0, and cy19:0, whereas a concomitant increase occurred in cy17:0 in both clover and ryegrass treatments between November and April (Figs. 5 and 6). Between April and June, there was a significant decrease in ryegrass straw-derived C in 16:0 and 18:1+, which was accompanied by a significant increase in straw-derived C in 18:2ω6,9 (Figs. 6 and 7).

3.3. Plant species effects on root-C incorporation into the soil microbial community

An NMS analysis of root derived-C data showed the percentage distributions of PLFA-C derived from both clover and ryegrass root C were significantly different from that of total PLFA-C ($p<0.01$, Fig. 8) in the September (Fig. 1), November (Fig. 5), and June sample (Fig. 7). In addition, the percentage distribution of PLFA-C derived from ryegrass root C was significantly different from that of total PLFA-C in October ($p<0.01$; Figs. 4 and 8). The PLFA that were most strongly correlated ($r>0.70$) with either NMS axis were 18:2ω6,9 and 18:1ω9 (axis 1) and 18:1+ (axis 2). The distribution of root-derived C in PLFA of clover and ryegrass treatments were significantly different from each other in September ($p<0.01$, Figs. 1 and 8), October ($p<0.05$, Figs. 4 and 8), and June ($p<0.01$, Figs. 7 and 8). Both residue treatments and time of sampling

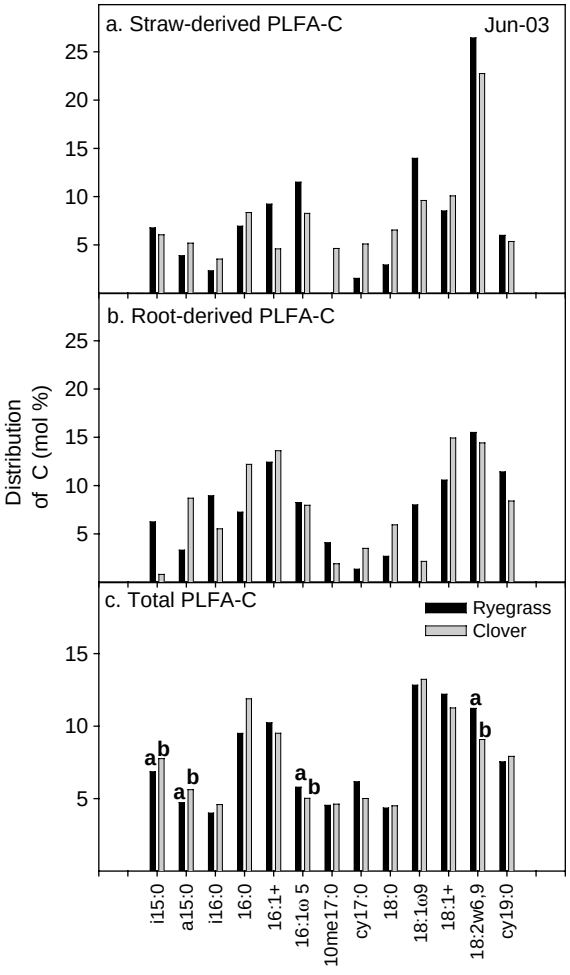

Fig. 7. The proportional (P_{Fi}) distribution of PLFA-C from straw (a), root-derived C (b), and the distribution of total (mol%) PLFA-C (c) sampled from soil in June 2002. Absence of vertical bars or designation by an asterisk denotes when a specific PLFA is not considered significantly different from zero. Small case letters designate significant differences between clover and ryegrass for that specific PLFA and sampling date ($n=4$; $p<0.01$).

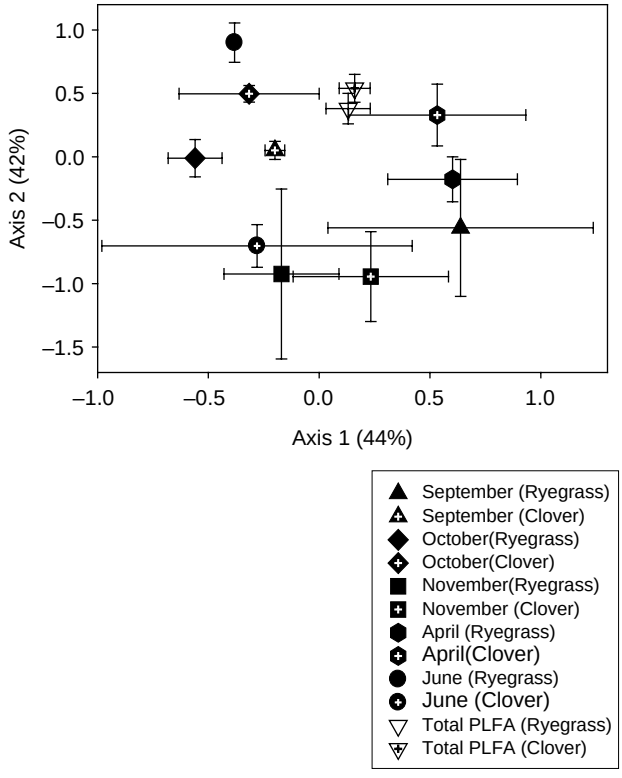

Fig. 8. The multivariate analysis of the proportional (P_{Fi}) distribution of root derived ^{13}C and total root-derived C in PLFA. The first and second axis using non-metric multidimensional scaling (NMS) are shown. Because the variability associated with mol% PLFA-C through the annual cycle was small compared to distribution of residue-derived PLFA-C, seasonal treatment means of mol% PLFA for clover and ryegrass were averaged for the purpose of NMS and multivariate statistical analysis. Percentage denote the amount of variability associated with each axis. Values of symbols and bars represent means and standard error, respectively (n=4).

contributed to the change in the relative amounts of root-derived C among the PLFA, and to the distribution of data in the NMS plot (Figs. 8). Neither the effect of residue treatment nor time of sampling grouped in a way that suggested that one of them was more responsible than the other for the spread among the data.

3.4. Influence of clover and ryegrass straw and root-derived C on soil microbial community structure

The mol% distribution of PLFA (data not shown) was influenced both by sampling time and treatment ($p<0.05$). In an NMS analysis, PLFA 18:2ω6,9 and a15:0 showed the strongest correlations with axis 1, and 18:1+ and 16:0 with axis 2 ($r>0.70$) (data not shown). Treatment differences between soils containing either clover or ryegrass residues were primarily due to statistically significant effects ($p<0.05$) occurring in April and June (Figs. 6 and 7). Indicator species analysis showed that the fungal biomarker, 18:2ω6,9, was the only PLFA that consistently separated treatments ($p<0.01$), with a greater mol% of 18:2ω6,9 in soil treated with ryegrass relative to clover residues in October, April, and June (Figs. 4, 6 and 7). Although the majority of PLFA showed significant temporal effects, no specific PLFA could account for the effect of sampling time based on indicator species analysis.

4. Discussion

4.1. Residue and seasonal influences on carbon flow within the microbial community

Although the interpretation of ^{13}C flow into microbial PLFA from ^{13}C-labeled plant residues is constrained by the complexity of residue composition, we gained some useful insights into the interaction between residue decomposition and time, and into the dynamics of the active members of

the microbial community through 9 months of diverse field conditions. One of the most important findings of this study was that C flowed differentially from straw and roots of clover and ryegrass residues into the soil microbial community. Residue-C found in any individual PLFA accounted for only a fraction of the total C in that particular PLFA, suggesting that many members of the soil community were either not involved in residue decomposition, or were simultaneously assimilating both soil and residue C, or, if they were mineralizing residue C, they were not growing and synthesizing PLFA. Several possibilities could account for this result. First, only a fraction of the soil microbial community are likely to possess the enzymes (e.g. cellulases and ligninases, etc.) necessary to degrade the residue-derived compounds. Secondly, soil mineral N levels were generally low throughout the study (≤ 4 μg $NH_4^+ + NO_3^-$–N g^{-1} soil), with the exception of a transient flush of mineral N seen in the clover treatment between October and November, so that microbial growth would have been N-limited during decomposition of structural plant polymers (Schimel and Weintraub, 2003). Indeed, Phillips et al. (2002) found that 10 times more ^{13}C from *N*-acetylglucosamine was recovered in PLFA than from ^{13}C cellobiose indicating that N can severely limit PLFA biosynthesis when N deficient substrates are added to soil. Thirdly, the soil community immobilizing residue C into PLFA might be limited to those organisms in close proximity to the residues. Gaillard et al. (1999) identified ^{13}C labeled straw-C in microbial biomass no further than 4-mm distance from the residues. During the early stage of residue decomposition (September–October), we noted that the water-soluble residue C component declined precipitously (data not shown), and may have been primarily responsible for the microbial growth that occurred in soil adjacent to residues during the early phase of decomposition (McMahon et al., 2005). It is well known that plant residues may contain up to 25% by weight of water-soluble materials (Swift et al., 1979), which decompose rapidly (Saviozzi et al., 1997), and can also influence decomposition of the water insoluble residue fraction (Reinertsen et al., 1984; Cogle and Saffigna, 1989).

Compared to the total soil microbial community, we also found that the active microbial community, as indicated by residue C flow into PLFA, was usually structurally distinct and more dynamic in response to both residue and season. Though it is not necessarily surprising that the structure of the actively metabolizing and whole-soil microbial communities differed, these results nevertheless provided a more detailed look at the activities of the microbial community separated from the mostly inactive total soil microbial community.

By identifying both the PLFA that incorporated little residue-derived C in the early decomposition period, along with the PLFA in which the percentage of residue derived C changed significantly between sampling times, we obtained insight into the dynamics of the soil microbial community associated with residue decomposition. For example, between September and October, ryegrass straw-derived C increased in more PLFA than did clover-derived C. This observation suggests that the substrate quality of the ryegrass residue selected for the growth of a more diverse soil community than did the clover straw residue. On the other hand, between October and November, there was large decline in total straw derived ryegrass PLFA-C and a significant re-allocation of residue derived C among the PLFA. These changes were related to the large reductions in PLFA-C associated with fungal biomass (18:1ω9; 18:2ω6, 9). Interestingly, the October–November period coincided with the first measurable decline in water insoluble components of the ryegrass residues (data not shown), whereas in the case of clover, this fraction had already been partially depleted during September–October. Certainly, the differences in the distribution of PLFA-C derived from residue that occurred between October and November could be attributed directly to differences in the chemistry of the residues. Further work is needed to determine if the dynamics occurring in the soil PLFA-C depend directly on the sequence of decompositional events occurring on the residues per se.

The winter period (November–April) was characterized by disappearance of many of the treatment differences seen among the soil PLFA fingerprints. In addition, during this period an increase was observed in the contribution of residue-derived C in some residue-associated microbial PLFA (data not shown). In this context, considerable interest has been shown in residue/litter decomposition under winter conditions in agricultural and native ecosystems, with particular emphasis on the relative sensitivities of N immobilization and N mineralization to low temperature (Clein and Schimel, 1995; Van Scholl et al., 1997; Ambus and Jensen, 2001). More work is needed to ascertain what kinds of microorganisms immobilize residue C during the winter months, and what impact they have on the properties of residue decomposition and nutrient cycling in general. In this context, previous studies showed incorporation of ^{13}C substrates into cyclopropyl fatty acids under anaerobic conditions, which was linked to growth of anaerobic microorganisms such as acetogens and sulfate-reducing bacteria (Reichardt et al., 1997; Pel et al., 1997; Pelz et al., 2001). Certainly, the relative accumulation of straw and root residue-derived C in cy17:0 between November and April fits with the above idea. It also remains a possibility that accumulation of cy17:0 reflects a response of community members to stress (White, 1993).

4.2. Dynamics of root-C within the soil microbial community

Although many studies have been conducted on the decomposition of straw residues, far less information is generally available about the decomposition of roots and the microbial communities involved (Gale et al., 2000; Puget and Drinkwater, 2001). The fact that root-derived C

contributed between 30 and 40% of soil PLFA-C at the initiation of the experiment in September, attests to the magnitude of C flow from plant roots into the soil community (Butler et al., 2003; 2004; Lu et al., 2004). Furthermore, it was intriguing to observe that although root-derived C in PLFA declined quickly upon soil rewet in the fall, a greater proportion of ryegrass root relative to clover root-derived C seemed to have stabilized in soil PLFA. Further work is needed to compare and contrast the soil communities that sequestered the root C, and where they are located in the soil fabric.

Several recent studies have reported that fungi are major sinks for newly added C based upon the incorporation of ^{13}C from various substrates into 18:2ω6,9 (Arao, 1999; Butler et al., 2003; Waldrop and Firestone, 2004). In this context, both 18:1ω9 and 18:2ω6,9 were identified as strong contributors to the seasonal dynamics of C flow throughout the growing season, and to differences between clover and ryegrass root residues. Based upon the ^{13}C content of these two PLFA in the September samples, fungi apparently sequestered a larger proportion of clover root C compared to ryegrass during the growing season and its aftermath. It remains unclear to what extent rhizodeposits during the growing season, versus root senescence during the period between seed set (June) and the time of soil excavation (September) supplied the root-C for fungal growth. Between September and November, and in the following spring samples (April and June) fungi clearly derived a greater proportion of root C from ryegrass compared to clover. Recent molecular studies have shown that phylogenetically distinct fungal populations can dominate soil at different times of the year (Gomes et al., 2003; Schadt et al., 2003). Further studies are needed to dissect the nature of the fungal communities associated with the seasonal dynamics of residue-C appearance in 18:2ω6,9.

4.3. Conclusions

Using an in situ field experiment we have demonstrated that soil microbial community dynamics and activity are at least partly dependent on the type of C that is made available for microbial catabolism. To what extent each of the concomitant dynamic changes in season or substrate chemistry influence the flow of C to the microbial community remains to be investigated. Although broad in scope, analysis of microbial dynamics using ^{13}C-PLFA and other compound specific isotope techniques provide important insights into the linkages between microbial community dynamics substrate quality and time during the residue decomposition.

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