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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