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REGULAR ARTICLE

Microbial community assimilation of cover crop rhizodeposition within soil microenvironments in alternative and conventional cropping systems

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Abstract
Background and Aim Root-derived carbon (C) is preferentially retained in soil compared to above-ground C inputs. Microbial communities in the rhizosphere are crucial to nutrient and organic matter cycling within agroecosystems. The overall aim of this study was to investigate the impacts of crop management on microbial community structure and processing of rhizodeposit-C within microenvironments of two soil zones, the rhizosphere versus non-rhizosphere.
Methods New root-C (C_{new}) from ^{13}C-labeled hairy vetch (*Vicia dasycarpa*) plants were traced into phospholipid fatty acids (PLFA) within microaggregate (53–250 μm) and silt-and-clay (<53 μm) microenvironments in rhizosphere and non-rhizosphere soil during the cover crop growing season in long-term conventional (annual synthetic fertilizer applications), low-input (synthetic fertilizer and cover crop applied in alternating years), and organic (annual composted manure and cover crop additions) maize-tomato systems (*Zea mays* L.- *Lycopersicum esculentum* L.).
Results Among the three cropping systems, the composition of the microbial communities processing root-derived C were similar, which implied that the cropping systems maintained diverse microbial communities that were capable of utilizing similar C substrates despite receiving different long-term nutrient inputs. Relative distributions of root-derived PLFA-C (^{13}C mol%) in the rhizosphere and non-rhizosphere were not significantly different, thereby suggesting that the structure of the microbial community utilizing new root-C in the rhizosphere- and non-rhizosphere microenvironments were similar. However, total PLFA biomass was four times greater, and root-derived PLFA-C in both soil microenvironments were approximately 10 times greater in the rhizosphere than in the non-rhizosphere. Although no microbial group dominated the processing of C_{new} in the microenvironments of the rhizosphere and non-rhizosphere, we found that the microbial community of the silt-and-clay in the rhizosphere played a different role in the cycling of C_{new} compared to communities in the rhizosphere microaggregates and those in the silt-and-clay and microaggregates of the non-rhizosphere.
Conclusions Our results confirm that rhizodeposition plays an important role in the greater contribution of root-C than residue-C to SOM stabilization. This

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study also showed that microbial communities assimilating rhizodeposit-C are sensitive to their microenvironment (i.e., microaggregates versus silt-and-clay particles); nevertheless, differences in long-term crop management did not lead to differences in the capacity of the microbial communities to utilize active cover crop root-C substrates.

Keywords ^{13}C-PLFA · Aggregates · Cropping systems · Microenvironments · Rhizosphere

Introduction

Carbon (C) input from plant roots can influence associated soil microbial communities (i.e., population density and diversity) through the types and amounts of C and nutrient inputs to the rhizosphere (Bowen and Rovira 1976; Kent and Triplett 2002; Paterson 2003). During the growing season, active roots are continuously exuding a range of organic compounds (i.e., mainly carbohydrates, carboxylic acids, and amino acids) directly into the rhizosphere (Michulmas et al. 1985; Oades 1978), which represent 15–33% of the C assimilated by crops and translates to approximately 0.9–3 Mg C ha^{-1} (Hailer and Stolp 1985; Kuzyakov and Schneckenberger 2004; Swinnen et al. 1994; Warembourg and Paul 1973). Within the mosaic of habitats in the soil, microorganisms preferentially colonize the rhizosphere because root exudates (i.e., sloughed cells, secretions and exudates) are a major source of nutrients in soils, making the rhizosphere an area of intense activity with specific biological, chemical, and physical characteristics (Kennedy 1998; Lynch and Whipps 1990). Conversely, microbial communities can affect rooting patterns, stimulate plant root growth (e.g., release of hormones, neutralizing toxic substances, etc.), and influence the supply of available nutrients for plant uptake (Barea et al. 2005; Bowen and Rovira 1999). Microbial turnover of rhizodeposits plays an important role in C flow through soils; yet, there is limited information on the structure and the rates of C processing, particularly in situ, associated with active rhizosphere microbial communities (Butler et al. 2003; Denef et al. 2009; Treonis et al. 2004).

The soil microbial community plays a crucial role in alternative agricultural systems (e.g., low-input and organic systems) that rely more on the internal cycling of nutrients through the soil microbial community than conventional systems, which are often maintained in a state without nutrient limitations and are inherently leaky as a result of surplus additions of nutrients (Drinkwater and Snapp 2007). Shifts in microbial growth and activity, such as increases in fungal abundances relative to bacterial populations, have been associated with increased C and nitrogen (N) retention in soil (Högberg et al. 2007; Six et al. 2006). A decrease in the functional diversity of the soil microbial community can subsequently impair enzyme activities related to N cycling (Kandeler et al. 1996). Therefore, to fully exploit the potential of alternative cropping systems, a better understanding of the structure and functioning of microbial populations in the rhizosphere and their succession during plant development is needed.

Both agricultural management practices (e.g., tillage, C additions, cropping rotation, and irrigation) (Bronick 2005; Elliott 1986) and rhizosphere processes (Angers and Caron 1998; Six et al. 2002) can heavily influence the physical structure (e.g., aggregation) of the soil environment. Miller and Jastrow (2000) showed that microbial activity in the rhizosphere contributes to the formation and stabilization of soil aggregates. Moreover, the dynamic nature of the rhizosphere-soil structure complex can create physicochemically varying microenvironments for soil microorganisms (Ranjard and Richaume 2001) and, consequently, determine the size, distribution, and activity of the microbial community among aggregates of different sizes (Beare et al. 1992; Frey et al. 1999; Schutter and Dick 2002). For example, less microbial biomass has been found in microaggregates (<250 μm) than in macroaggregates (>250 μm) (Gupta and Germida 1988; Miller and Dick 1995). Also, several studies have shown a spatial compartmentalization of bacteria among aggregates (Elliott et al. 1980; Kuikman et al. 1990; Vargas and Hattori 1986). It is still unclear, however, how changes in microbial decomposer community composition and activity within different soil microenvironments can affect soil organic matter (SOM) stabilization and long-term soil organic C sequestration.

The preferential retention and greater contribution of root-derived carbon to SOM-C has been reported in several studies (e.g., Balesdent and Balabane 1996; Kong and Six 2010; Rasse et al. 2005). It is estimated

that 2–4% of C from rhizodeposition, e.g., root exudates and other organic substances released by plant roots during plant growth, remains adsorbed on clay minerals and SOM, while 0.8–3.2% is incorporated into rhizosphere microorganisms (Kuzyakov and Domanski 2000; Van Ginkel et al. 2000). In Kong et al. (2011), we addressed the relationship between long-term C sequestration at the cropping system-scale and C cycling within microbial groups in soil aggregates by comparing the distribution of C from above- and belowground hairy vetch (*Vicia dasycarpa*) into the microbial communities after the cover crop growing season. Estimates of root-C in Kong et al. (2011) included both rhizodeposition from the cover crop growing season and C from root decomposition after the cover crop was incorporated. In the current study, we sought to elucidate the mechanisms governing the in-season rhizodeposition contribution to SOM-C, which was not partitioned from C derived from root decomposition in the estimates of SOM derived from root-C in Kong et al. (2011).

The overall objective of this study was to investigate the impacts of crop management on the structure of and rhizodeposit-C processing (i.e., C incorporation into microbial biomass and/or deposition or respiration of C) within microbial communities in microenvironments of two soil zones, rhizosphere versus non-rhizosphere soil. Hairy vetch root-C was ^{13}C-labeled and traced into the phospholipid fatty acids (PLFAs) of microorganisms within soil microenvironments (i.e., microaggregates and silt-and-clay particles) in long-term conventional, low-input, and organic maize-tomato cropping systems to link microbial community structure to the short-term processing of rhizodeposit-C during the cover crop growing season. The following specific hypotheses were tested: (i) more C is processed by soil microbial communities in rhizosphere than non-rhizosphere soil, (ii) the first-time growth of vetch in the conventional system will result in less PLFA biomass, a different microbial community structure, and slower rates of microbial C processing within the rhizosphere of the conventional than in the low-input and organic systems, and (iii) the microbial community in the rhizosphere-microaggregate processes more C than microbial communities in the non-rhizosphere-microaggregate and both the rhizosphere- and non-rhizosphere-silt-and-clay.

Materials and methods

Study site, experimental design, and field operations

This field study took place during the winter 2006–2007 cover crop growing season in the Long Term Research on Agricultural Systems (LTRAS) plots at the Russell Ranch experimental site (Davis, CA, USA; 38°32′24″ N 121°52′12″ W). The LTRAS plots are located in a Mediterranean climate region (i.e., wet winters and hot, dry summers) and are established on two similar soil types: i) Yolo silt loam (fine-silty, mixed, nonacid, thermic Typic Xerorthent) and ii) Rincon silty clay loam (fine, montmorillonitic, thermic Mollic Haploxeralf). Three maize-tomato (*Zea mays* L.- *Lycopersicum esculentum* L.) cropping systems (n=3) were compared: conventional (annual synthetic fertilizer applications); low-input (synthetic N fertilizer applied in alternate years with cover crop-N incorporated the years without synthetic N fertilization); and organic (annual composted manure and cover crop additions). Since 1993, these cropping systems have been arranged in a completely randomized design with three 0.4 ha-replicates that received furrow irrigation and were conventionally tilled.

The conventional system received 51 kg N ha^{-1} as N-P-K starter and 170 kg N ha^{-1} as ammonium nitrate side-dressing, while composted manure (373 kg N ha^{-1}) and winter cover crop residues were incorporated into the organic cropping system. Fertilizer applications to the low-input system comprised of 161 kg N ha^{-1} during the tomato rotation, alternating with the growth and incorporation of winter cover crop residue before the maize phase of its two-year rotation.

$^{13}CO_2$ pulse-labeling of hairy vetch

At the start of the cover crop growing season (mid-November 2006), hairy vetch seeds were broadcast-sown into 5×5 m microplots within each of the cropping systems. On February 19 (96 days after planting [DAP]), March 8 (113 DAP), March 24 (129 DAP), and April 10 (146 DAP), 2007, a 720 cm^2 area in each of the treatment replications was ^{13}C-labeled by injecting 0.3, 0.6, 0,8, and 1.1 L ^{13}C-CO_2, respectively, into cylindrical plexiglass chambers (30 cm diameter; Interstate Plastics, Sacramento, CA). At each labeling event, the labeling chambers were placed over vetch shoots and inserted into the soil to a depth of

approximately 5 cm. The ^{13}C-labeling procedure used in this study was adapted for the cylindrical labeling chamber from methods described in more detail in Kong et al. (2011). Briefly, ^{13}C-CO_2 (99 atom%) was injected into the ^{13}C-labeling chamber; the lid was removed when the CO_2 level in the chamber dropped below 250 ppm; the lid was placed once more over the vetch plants at sunset to capture overnight $^{13}CO_2$ respiration, consequently improving ^{13}C assimilation efficiency; the lid was removed the following morning after CO_2 levels in the chamber dropped below 250 ppm. After the labeling chambers were removed (approximately 24 h after the $^{13}CO_2$ injection), the ^{13}C-labeled vetch shoots were collected, 30 cm diameter polyvinylchloride (PVC) cores were inserted to a depth of 30 cm below the base of the vetch shoots, and the rhizosphere soil cores were extracted.

On April 2, 2007 (132 DAP), unlabeled vetch shoots and rhizosphere cores were collected from each treatment replicate, in the same manner described above, to establish δ^{13}C natural abundance values for the measured variables.

Vetch shoots and rhizosphere soil processing

Aboveground vetch shoots collected after each ^{13}C-labeling event were weighed and dried, to determine dry matter content, and then subsampled for elemental and isotopic C concentrations. In each cropping system, aboveground vetch biomass was greater at 146 DAP than at 96 DAP (0.9 and 0.5 kg dry biomass m^{-2}, respectively). The rhizosphere soil cores were stored at 4°C until they were separated into rhizosphere and non-rhizosphere soil. *Rhizosphere* soil was obtained by shaking the mass of vetch roots and collecting soil adhering to the roots. Soil that did not adhere to the roots was considered *non-rhizosphere* soil; however, it was impossible to remove roots from the non-rhizosphere soil completely. Visual criteria (e.g., color and elasticity) were used to distinguish hairy vetch roots from non-hairy vetch roots. Once collected, ~10 g of both rhizosphere and non-rhizosphere soils were sub-sampled for moisture content and the remainder were stored at −20°C until fractionation.

After thawing for 20 min at 4°C, ~30 g subsamples of rhizosphere and non-rhizosphere soils were fractionated into three aggregate size classes, coarse particulate organic matter (cPOM: >250 μm), microaggregate (53–250 μm), and silt-and-clay (<53 μm), using a microaggregate isolator according to the methodology outlined in Six et al. (2000). Thawed soil samples were immersed in deionized water on top of a 250 μm mesh screen and gently shook with 50 stainless steel beads (4 mm diameter) until only cPOM and sand were retained on the 250 μm mesh screen. During shaking, a continuous and steady stream of water flowed through the device to ensure that microaggregates were immediately flushed onto a 53 μm sieve and were not exposed to any further disruption by the beads. The material on the 53 μm sieve was manually sieved for 2 min by moving the sieve 50 times, in an up-and-down motion (Elliott 1986), to separate water-stable microaggregates from silt-and-clay particles. All fractions were collected as separate soil suspensions, which were centrifuged at 5,000 rpm for 15 min at 4°C (Sorvall RC-5C Plus Superspeed centrifuge, Thermo Scientific). The supernatant was discarded and the remaining fractions were lyophilized and stored at −20°C until further analysis. For the rhizosphere soils, the cPOM predominantly consisted of roots associated with the rhizosphere and, thus, were used for reference δ^{13}C values of root-C input. For more details on the procedure used to isolate aggregate fractions from rhizosphere and non-rhizosphere soil, see Kong and Six (2011).

Subsamples of vetch shoots, cPOM, and soil aggregates were ground and analyzed for elemental and isotopic C concentrations using a PDZ Europa ANCA-GSL isotope ratio mass spectrometer (Sercon, Crewe, UK). Carbon isotope ratios (δ^{13}C) were expressed per mil (‰) using:

$$\delta^{13}C = \left[\left(\frac{^{13}R_{sample}}{^{13}R_{standard}}\right) - 1\right] \times 1000 \quad (1)$$

where $^{13}R = {}^{13}C/{}^{12}C$ and the standard is Pee Dee Belemnite (PDB).

At 96 and 146 DAP, the δ^{13}C values of the vetch shoots were ~59‰ and 135‰, respectively, whereas the roots had enrichments of ~−8‰ and −3‰, respectively. Among the cropping systems, δ^{13}C values for the shoots and roots did not significantly differ. Natural abundance values of unlabeled vetch shoots and roots were −30 and −20‰, respectively.

PLFA extraction, quantification, and ^{13}C analysis

Phospholipid fatty acids (PLFAs) were extracted from the microaggregates and silt-and-clay from rhizosphere and non-rhizosphere soils according to the procedure of Bossio and Scow (1998) and described in detail in Kong et al. (2011). Briefly, total lipids were extracted from 4 g of lyophilized sample using a solution of potassium phosphate buffer, chloroform, and methanol. Phospholipids were then fractionated from neutral and glycolipids on a silicic acid column (Supelco, Inc., Bellfonte, PA), and then subjected to mild alkaline methanolysis to form fatty acid methyl esters (FAMEs), which were analyzed with a gas chromatography-combustion-isotope ratio mass spectroscopy (GC-c-IRMS) system (Thermo Electron Corp., Bremen, Germany). Individual FAME peaks were identified by comparison with the following standards: a mixture of 37 FAMEs (FAME 37 47885-4; Supelco, Inc.), a mixture of 24 bacterial FAMEs (P-BAME 24 47080-U; Supelco, Inc.), and a MIDI FAME standard (Microbial ID, Inc.). Chromatographic peaks were quantified by comparison to peaks of an internal standard [nonadecanoic FAME (19:0)]. Total PLFA biomass was quantified as the total sum of extracted PLFAs (nmol g^{-1} dry soil; Frostegård and Bååth 1996).

Enrichment values of individual FAMEs were corrected for the C atoms introduced during the addition of the methyl groups during transesterification by mass balance:

$$\delta^{13}C_{PLFA} = \frac{[(N_{PLFA}+1)]\delta^{13}C_{FAME} - \delta^{13}C_{MeOH}}{N_{PLFA}} \quad (2)$$

where N_{PLFA} refers to the number of C atoms of the PLFA, $\delta^{13}C_{FAME}$ is the $\delta^{13}C$ value of the FAME after transesterification, and $\delta^{13}C_{MeOH}$ refers to the $\delta^{13}C$ value of the methanol used for the transesterification (−27.2±2.9‰).

Standard fatty acid/PLFA nomenclature (A:BωC) was used and is as follows: 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 groups, cyclopropane groups, and iso- and anteiso-branched fatty acids, respectively. 'Cis' and 'trans' geometry are indicated by the suffixes 'c' and 't', respectively.

Of 40 FAME peaks on average that were detected in each sample, 25 FAMEs were present in abundances greater than 0.5 mol% (mole percentage) for both ^{13}C-PLFA and total PLFA C (~97% of the total PLFA concentration) and, therefore, in sufficient quantity to obtain accurate $\delta^{13}C$ values. Fatty acids 18:1ω9t and 18:1ω7c were not resolved and eluted as one peak (designated 18:1ω7), as did 18:1ω9 and 18:3ω3 (designated 18:1ω9). Phospholipid fatty acid biomarkers were assigned to four microbial groups: Gram-positive bacteria–*i*15:0, *a*15:0, *i*16:0, *i*17:0, *a*17:0 (Zelles 1999); Gram-negative bacteria–18:1ω7, *cy*17:0, *cy*19:0 (Zelles 1999); fungi–18:2ω6 and 18:1ω9 (Frostegård and Bååth 1996; Zelles 1997); actinomycetes–10Me16:0 and 10Me18:0 (Zelles 1999). Phospholipid fatty acids not assigned as biomarkers (12Me16:0, 14:0 3-OH, 16:1 2-OH, 15:0, 16:0, 18:0, *i*14:0, 16:1ω5, 16:1ω9c, 16:1ω9t, 10Me17:0, 17:1, and 18:3ω6) were included in total PLFA yields. Fungal: bacterial ratios were calculated by dividing the sum of fungal PLFA biomass (nmol g^{-1} soil) by the sum of Gram-positive and Gram-negative bacterial PLFA biomass (Zelles 1999).

The proportion (*f*) of PLFA-C in the soil microenvironments derived from ^{13}C-labeled root-C was calculated as follows:

$$f = \frac{{}^{13}C_{PLFA} - {}^{13}C_{natural\ abundance\ PLFA}}{{}^{13}C_{labeled\ material} - {}^{13}C_{natural\ abundance\ PLFA}} \quad (3)$$

where ${}^{13}C_{PLFA}$ = $\delta^{13}C$ for the PLFA of interest, ${}^{13}C_{labeled\ material}$ = $\delta^{13}C$ of the root-C, and ${}^{13}C_{natural\ abundance\ PLFA}$ = $\delta^{13}C$ of the equivalent PLFA from the unlabeled rhizosphere core (natural abundance). Total PLFA-C concentrations in the soil microenvironments were multiplied by *f* to obtain *PLFA-C*$_{new}$, i.e., the concentration of PLFA-C derived from ^{13}C-labeled root-C (nmol g^{-1} dry soil). Data were also expressed as ^{13}C mole percentages (^{13}C mol%), which were calculated as the PLFA-C_{new} of each PLFA peak relative to the summed PLFA-C_{new} of all PLFA peaks in the sample.

Data and statistical analyses

An initial repeated measures analysis performed for all variables across 96, 113, 129, and 146 DAP did not

show a significant effect of time; consequently, data from all four sampling events were not further analyzed together. Data from 96 to 146 DAP were analyzed as a split-plot, completely randomized design using the PROC MIXED procedure of the Statistical Analysis System (SAS; SAS Institute 2002) with the main effects of cropping system, soil zone (rhizosphere or non-rhizosphere), soil microenvironment, sampling event, and their interactions treated as fixed effects and plot considered a random effect. Differences between means were calculated based on least significant difference tests, with the PDIFF option of the LSMEANS statement. Letters for mean separation in PROC MIXED were assigned using the macro PDMIX 800 (Saxton 1998). All differences discussed were significant at the $p<0.05$ probability level, unless otherwise stated. Total PLFA biomass measurements were transformed to their natural logarithms to account for standard deviations being proportional to the means.

The PLFA-C_{new} data were analyzed with principal component analysis (PCA). This multivariate community analysis was performed on individual PLFA-C_{new} concentrations using CANOCO version 4.0 (Microcomputer Power, Inc., Ithaca, NY).

Results

Carbon content and distribution of soil microenvironments

Data in Table 1 show differences associated with a four-way sampling event x soil zone x cropping system x soil microenvironment interaction. The silt-and-clay fraction from the rhizosphere of the organic system at 96 DAP had the greatest C content (15.7 g C kg soil^{-1}) among the microenvironments; while microaggregates from non-rhizosphere soils in the conventional system contained the lowest C content of the microenvironments at 96 and 146 DAP (6.7 g C kg soil^{-1}; Table 1). With the exception of microaggregates from the low-input system (146 DAP) and from the conventional system (96 and 146 DAP), C contents in the microenvironments were greater in the rhizosphere than in the non-rhizosphere.

At both 96 and 146 DAP, the silt-and-clay microenvironment constituted between 50 and 63% of the whole soil, while the microaggregates comprised 34–42% of the whole soil and the distribution of microenvironments did not differ across the cropping systems (Table 2). While the percentages of microaggregates in rhizosphere versus non-rhizosphere soil were not different at both 96 and 146 DAP, the proportion of silt-and-clay in non-rhizosphere soil at 96 and 146 DAP (61.4 and 63.0%, respectively) were greater than in rhizosphere soil (53.6 and 50.0%, respectively; Table 2). The proportion of rhizosphere-microaggregates decreased from 96 DAP (41.7%) to 146 DAP (33.8%); whereas, the proportion of non-rhizosphere microaggregates were not different from 96 to 146 DAP.

PLFA biomass and relative abundance of microbial biomarkers

A soil microenvironment x soil zone interaction was observed for PLFA biomass (nmol PLFA g^{-1} dry soil) at both 96 and 146 DAP (Fig. 1). Phospholipid fatty

Table 1 Total carbon content (g C kg soil^{-1}) of soil microenvironments [microaggregate (53–250 μm) and silt-and-clay (<53 μm)] across the conventional, low-input, and organic cropping systems 96 and 146 days after planting (DAP). Differences associated with the carbon content of soil microenvironments at 96 and 146 DAP are indicated with different letters within parentheses ($p<0.05$)

Days after planting	Soil microenvironment	Rhizosphere			Non-Rhizosphere		
		Conventional	Low-input	Organic	Conventional	Low-input	Organic
		(g C kg soil^{-1})					
96	microaggregate	9.0 (ef)	12.6 (c)	12.8 (c)	6.7 (g)	8.1 (f)	11.9 (cd)
	silt-and-clay	12.6 (c)	13.1 (bc)	15.7 (a)	10.6 (d)	10.8 (d)	10.2 (d)
146	microaggregate	8.2 (f)	10.2 (d)	14.9 (ab)	6.7 (g)	9.2 (ef)	9.8 (de)
	silt-and-clay	12.7 (c)	13.4 (bc)	14.2 (b)	9.9 (de)	9.7 (e)	11.0 (d)

Table 2 Distribution of soil microenvironments (% of total soil mass) and relative abundances (%) of biomarkers in the microaggregate (53–250 μm) and silt-and-clay (<53 μm) microenvironments averaged across the conventional, low-input, and organic cropping systems 96 and 146 days after planting (DAP). Differences associated with the distribution of soil microenvironments at 96 and 146 DAP are indicated with different letters within parentheses ($p<0.05$). Within each sampling event (96 or 146 DAP), biomarker percentages followed by different upper case letters and different lower-case letters indicate significant differences at the $p<0.05$ level for 'Rhizosphere-' and 'Non-Rhizosphere-' derived biomarkers, respectively

Days after planting	Soil microenvironment	Rhizosphere					Non-Rhizosphere				
		Biomarkers (%)					Biomarkers (%)				
		Distribution (%)	Gram (+)	Gram (−)	Fungi	Actinomycetes	Distribution (%)	Gram (+)	Gram (−)	Fungi	Actinomycetes
96	microaggregate	41.7 (c)	21.5^{A}	17.8^{B}	9.4^{C}	5.0^{D}	37.1 (cd)	27.5^{a}	13.5^{c}	7.6^{d}	6.7^{d}
	silt-and-clay	53.6 (b)	17.4^{B}	17.6^{B}	5.8^{D}	3.6^{E}	61.4 (a)	23.9^{b}	16.0^{c}	7.2^{d}	7.4^{d}
146	microaggregate	33.8 (d)	21.6^{U}	19.4^{V}	10.1^{X}	4.7^{Z}	35.8 (d)	25.1^{w}	15.4^{y}	8.4^{z}	6.7^{z}
	silt-and-clay	50.0 (b)	15.8^{W}	24.4^{T}	6.8^{Y}	3.8^{Z}	63.0 (a)	19.7^{x}	16.1^{y}	7.0^{z}	7.5^{z}

acid biomass in the silt-and-clay microenvironment of the rhizosphere was over 2.5 times greater than PLFA biomass in the rhizosphere-microaggregates and the microenvironments in the non-rhizosphere at 96 DAP. Similarly, PLFA biomass in the rhizosphere-silt-and-clay at 146 DAP was nearly four times greater than the other three soil microenvironments across the cropping systems. Cropping system had neither a main nor interactive effect on PLFA biomass at either 96 or 146 DAP.

No cropping system effect was found for relative abundances of the microbial biomarker groups (nmol biomarker g^{-1} dry soil/total nmol biomarker g^{-1} dry soil, as a percentage) at 96 and 146 DAP. A soil microenvironment x microbial biomarker group interactive effect, however, was found within the soil zones at 96 and 146 DAP (Table 2). Gram-positive bacterial biomarkers dominated the PLFA biomass in both rhizosphere and non-rhizosphere soil at both 96 and 146 DAP, with the exception that Gram-negative bacteria in the rhizosphere-silt-and-clay at 146 DAP had the highest relative abundance (Table 2). In contrast, fungal and actinomycete biomarkers in both microenvironments comprised the lowest proportion of the total PLFA biomass within rhizosphere and non-rhizosphere soil at both 96 and 146 DAP. In non-rhizosphere soil, relative abundances of fungal and actinomycete biomarkers within the microaggregate and silt-and-clay

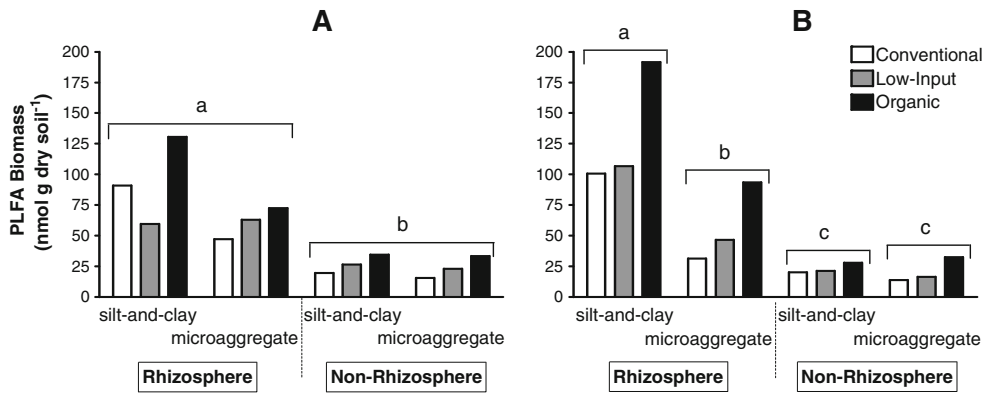

Fig. 1 Total phospholipid fatty acid (PLFA) biomass (nmol g^{-1} dry soil) 96 (**a**) and 146 days after planting (**b**). *Upper and lower-case letters* above bars indicate significant differences for a soil microenvironment x soil zone interaction at the $p<0.05$ level within the 96 and 146 days after planning sampling events, respectively ($n=3$)

microenvironments were similar. On the other hand, relative abundances of fungal biomarkers were higher than actinomycete biomarkers in rhizosphere soil and were generally higher in the microaggregates than in the silt-and-clay microenvironment (except at 146 DAP where the actinomycetes made up a similar proportion of the PLFA biomass in the microaggregate and silt-and-clay). Relative abundances of Gram-positive bacterial biomarkers in the microaggregate were greater than that in silt-and-clay microenvironments within both the rhizosphere and non-rhizosphere at 96 and 146 DAP. Relative abundances of fungal biomarkers in the microaggregates were also higher than in silt-and-clay at 96 and 146 DAP (62 and 47% higher, respectively), but only in the rhizosphere. Relative abundances of fungal biomarkers in non-rhizosphere soil at both 96 and 146 DAP were not different between the silt-and-clay and microaggregate microenvironments. Furthermore, the ratio of fungal:bacterial PLFA biomass was higher in the rhizosphere-microaggregates (0.24) than in non-rhizosphere-microaggregates (0.21) and the silt-and-clay of both rhizosphere and non-rhizosphere soils (0.20 and 0.18, respectively), the latter three of which were not different.

Root-derived PLFA-C in rhizosphere and non-rhizosphere soil

At 96 and 146 DAP, recoveries of ^{13}C-PLFA in the rhizosphere soil ranged from 1.7 to 334 nmol PLFA-C_{new} g^{-1} soil, whereas ^{13}C-PLFA recoveries in non-rhizosphere soil ranged from 0.23 to 32.4 nmol PLFA-C_{new} g^{-1} soil (Figs. 2 and 3). Interactions between soil zone x cropping system x soil microenvironment x microbial biomarker were significant at both 96 and 146 DAP (Figs. 2 and 3, respectively). New root-C incorporation was greatest into Gram-positive bacterial biomarkers in the silt-and-clay of the rhizosphere in the organic system at 96 and 146 DAP (262.2 and 334.0 nmol PLFA-C_{new} g^{-1} soil, respectively). Incorporation of rhizodeposit-C into fungal and Gram-negative and Gram-positive bacterial biomarkers of the rhizosphere were nearly 10 times greater than into PLFA biomarkers in the non-rhizosphere at 96 DAP (Fig. 2). A similar trend was observed at 146 DAP (Fig. 3). At 146 DAP, ^{13}C incorporation into fungal and Gram-positive biomarkers in rhizosphere soil decreased across the cropping systems in the following order: organic > low-input > conventional. In contrast, no differences in C_{new} were detected among the microbial biomarkers of non-rhizosphere soil across the three cropping systems at either 96 or 146 DAP.

The relative incorporation of root-derived C into microbial biomarker groups of the rhizosphere versus non-rhizosphere (i.e., ratio of the sum of PLFA-C_{new} within a biomarker group extracted from rhizosphere soil to the sum of PLFA-C_{new} within a biomarker group extracted from non-rhizosphere soil), ranged from 0.73 to 24.9 (data not shown), wherein a ratio having a value greater than '1' indicates the degree to which the rhizosphere receives more root-derived C into a microbial biomarker than the non-rhizosphere. A soil microenvironment x microbial biomarker interaction was observed for this ratio. Across cropping systems and sampling events, ratios were highest for the Gram-positive biomarkers in the silt-and-clay (14.8) and fungal biomarkers in the microaggregates and silt-and-clay microenvironments (15.4 and 13.4, respectively). Ratios for actinomycete biomarkers associated with silt-and-clay and microaggregates (ratio = 4.1 and 4.0) and Gram-negative biomarkers associated with the silt-and-clay microenvironment (ratio = 4.1) were approximately 3.5 times smaller than the ratios found for the Gram-positive and fungal biomarkers.

Microbial community composition associated with root-C assimilation into the rhizosphere- and non-rhizosphere

Both soil microenvironment and soil zone influenced the ^{13}C-PLFA profiles, whereas cropping system and sampling event did not exert a strong impact. New root-C incorporated into the microbial community separated along the continuum of the first axis, which explained 92.3% of the variance in the data, while 3.4% of the variance was represented along the second axis (Fig. 4). Across the cropping systems, PLFA-C_{new} taken from silt-and-clay of the rhizosphere at 96 and 146 DAP had more positive score values for the 1st principal component than PLFA-C_{new} taken from the microaggregate of the rhizosphere. The 1st principal component, however, did not clearly differentiate between PLFA-C_{new} associated with silt-and-clay and microaggregates of non-rhizosphere soil.

Because a main cropping system effect was not observed, the soil microenvironment x sampling event

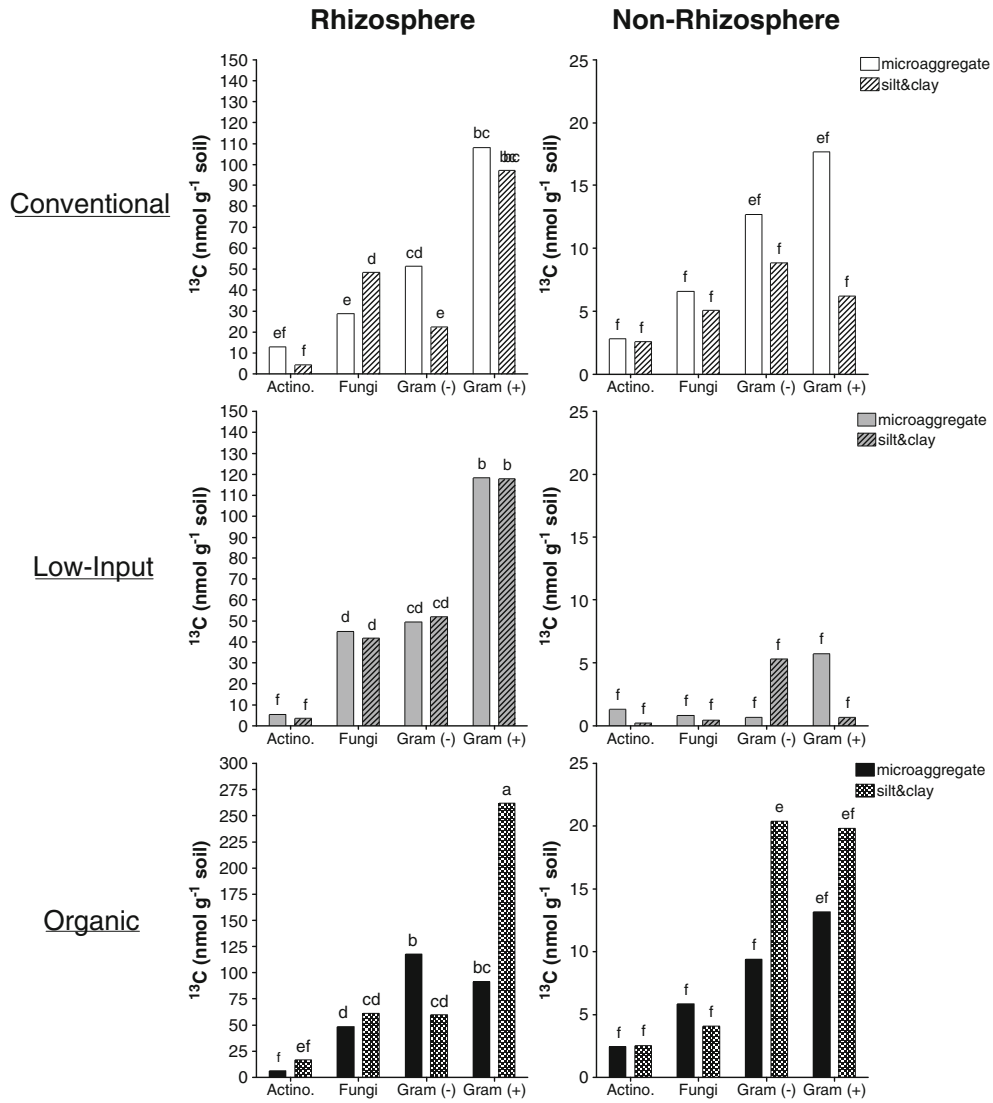

Fig. 2 Concentration of ^{13}C-PLFA biomarkers (nmol g^{-1} dry soil) extracted from the soil microenvironments (microaggregates and silt-and-clay) in rhizosphere (*left panel*) and non-rhizosphere soil (*right panel*) from the conventional, low-input, and organic cropping systems 96 days after planting. *Letters* above bars indicate significant differences at the $p<0.05$ level ($n=3$). Note differences in scales for the Y-axes. Actinomycetes is abbreviated as 'Actino'

x soil zone x microbial biomarker interactions for the relative assimilation of new root-C (expressed as ^{13}C mole percentage: ^{13}C mol%) into microbial biomarkers for only the low-input system (intermediate N input) at 96 and 146 DAP are discussed (Fig. 5). At both 96 and 146 DAP, the structure of the microbial communities actively utilizing new root-C in the rhizosphere- and non-rhizosphere were not significantly different. Gram-positive bacteria extracted from the silt-and-clay of non-rhizosphere soil at 96 DAP had the highest ^{13}C mol%, which indicates preferential utilization of root-C by Gram-positive bacteria in the silt-and-clay compared to the microaggregate fraction of the non-rhizosphere. No differences in ^{13}C mol% for the actinomycete biomarkers were observed between the sampling events and soil zones. In the rhizosphere, relative assimilation of root-derived C into the microaggregates and silt-and-clay microenvironments were not different at either 96 or 146 DAP.

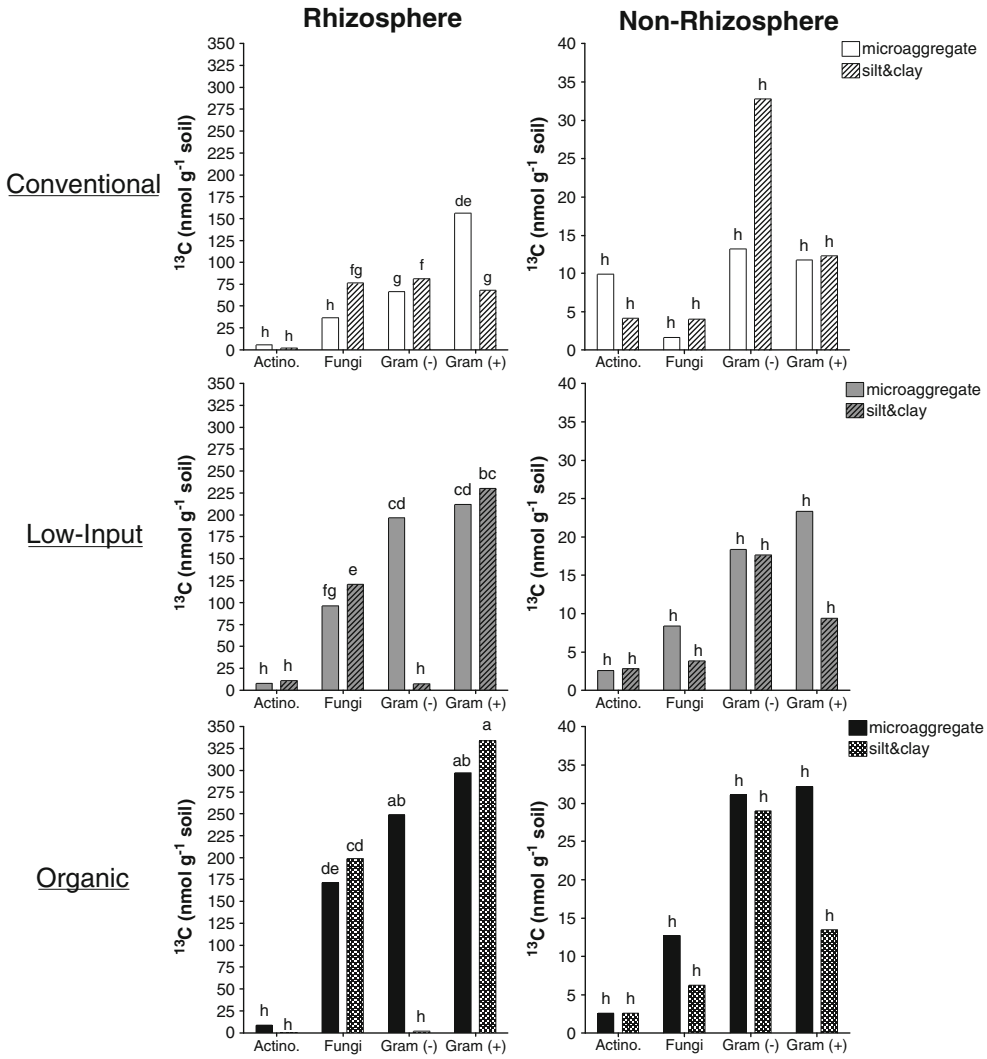

Fig. 3 Concentration of ^{13}C-PLFA biomarkers (nmol g^{-1} dry soil) extracted from the soil microenvironments (microaggregates and silt-and-clay) in rhizosphere (*left panel*) and non-rhizosphere soil (*right panel*) from the conventional, low-input, and organic cropping systems 146 days after planting. *Letters* above bars indicate significant differences at the $p<0.05$ level ($n=3$). Note differences in scales for the Y-axes. Actinomycetes is abbreviated as 'Actino'

Discussion

Rhizosphere versus non-rhizosphere effects on microbial community structure and C processing

Our first objective was to compare the root-C processing within microbial communities of the rhizosphere versus non-rhizosphere. Recoveries of ^{13}C-PLFA extracted from non-rhizosphere and rhizosphere soil were two to five orders of magnitude higher than recoveries of root-C from a growing leek crop (~0.01 nmol C g^{-1} soil: estimated from data in Elfstrand et al. 2008). We suggest that the higher ^{13}C-PLFA recoveries in our study compared to Elfstrand et al. (2008) can be attributed both to (i) the shorter duration between ^{13}C labeling and soil core sampling (~24 versus 48 h after the ^{13}C pulse) and (ii) potentially more root biomass, hence more rhizodeposits, of hairy vetch plants compared to leek plants. Finding nearly 10 times, on average, more PLFA-C derived from rhizodeposits in the rhizosphere than non-rhizosphere supported our hypothesis that more root-C was processed within soil microbial

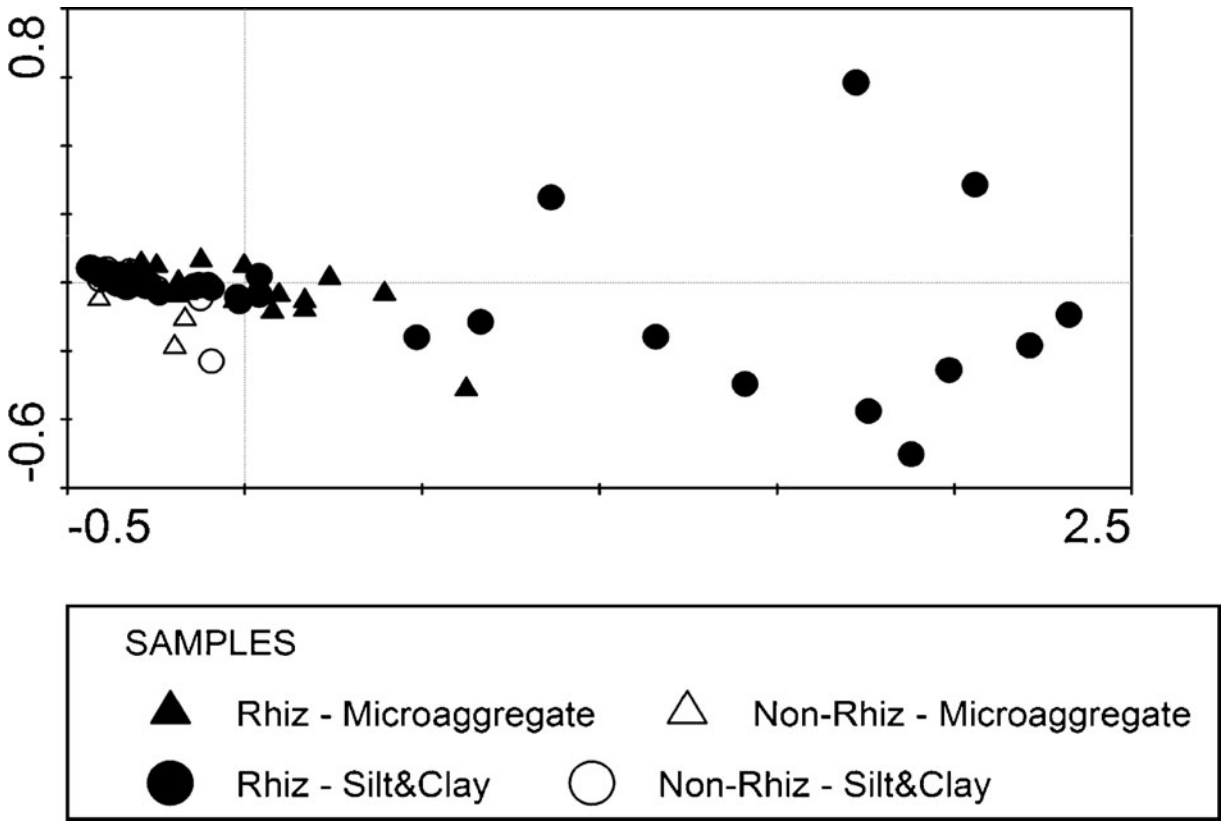

Fig. 4 Principal component analysis (PCA) of ^{13}C-PLFA extracted from microaggregate and silt-and-clay microenvironments in rhizosphere and non-rhizosphere soil collected 96 and 146 days after planting from the conventional, low-input, and organic cropping systems. Canonical axis 1 (x axis) explains 92.3% of the variance in the data, while canonical axis 2 (y axis) explains an additional 3.4% of variance in the data

communities in rhizosphere than non-rhizosphere soil. Our data agrees with Lu et al. (2007) who also found ^{13}C incorporation into PLFAs sharply decreased with distance away from rice roots. In a previous study on the same cropping systems, we found greater retention of root-C than residue-C despite incorporating five times more residue-C than root-C into the systems (Kong and Six 2010). Because root-C input during cover crop growth could not be distinguished from root-C input from decomposing roots in Kong et al. (2011), data in this current study provides evidence that rhizodeposition from the hairy vetch may have been a major source of the greater root-C contributions to SOM.

Studies employing ^{13}C-PLFA to investigate rhizosphere-C flow into microbial communities have reported assimilation of C from rhizodeposits into various microbial groups. High ^{13}C-enrichment of the fungal biomarkers, 18:1ω9 and 18:2ω6,9, and the non-specific PLFAs, 16:0 and 16:1ω5, observed by Treonis et al. (2004) suggested that a major pathway of C flux from grassland roots into soil microbial biomass involved fungi, particularly mycorrhizal fungi. Denef et al. (2009) also found fungal populations to be dominant in the processing of new rhizodeposits in grasslands. Meanwhile, Butler et al. (2003) showed that fungi and Gram-negative bacteria were most active in assimilating root-derived C in ryegrass soils. Lu et al. (2007) found relatively higher ^{13}C-labeling of 16:1ω9, 18:1ω7, 18:1ω9, 18:2ω6,9, and *i*14:0 PLFAs in the rhizosphere of rice, indicating that Gram-negative and eukaryotic microorganisms were most actively assimilating root-derived C, while *i*15:0 and *i*17:0 (Gram-positive biomarkers) were ^{13}C-enriched in the bulk soil. Our study found that Gram-positive bacteria played a larger role in total rhizodeposit-C incorporation both in the rhizosphere- and non-rhizosphere of a cover crop. Taken together, these results from ^{13}C-PLFA studies in grasslands and agricultural systems suggest that the

composition of the microbial community utilizing root-C may be shaped by ecosystem type; that is, bacteria (Gram-positive and Gram-negative) may play a larger role in cycling rhizodeposits in agricultural soils and fungi might be more active in grasslands. Dominance of root-C processing by fungi versus bacteria may have implications for soil C stabilization (Bardgett and McAlister 1999; Six et al. 2006); Six et al. (2006) suggested a quantitative and qualitative improvement of SOM, that had the potential to also enhance soil C sequestration, in agroecosystems favoring a fungal-dominated community due to the potentially higher efficiency with which fungi utilize C substrates and the recalcitrant nature of fungal byproducts in comparison to bacterial byproducts.

Although rhizodeposits stimulated the rates of C processing in the rhizosphere compared to the non-rhizosphere, the similarities in ^{13}C mol% associated with the rhizosphere- and non-rhizosphere suggested that, when corrected for the amount of C incorporated, the structure of the microbial communities assimilating root-C in the rhizosphere and non-rhizosphere were not different (Fig. 5). In a study on ryegrass, Butler et al. (2003) also did not find differences in mole percentages between individual PLFA extracted from rhizosphere and bulk soils. Balser et al. (2002) also reported that process rates were not accompanied by detectable changes in community composition. Some studies have suggested that differences in plant species and composition select for taxonomic and functional groups in the rhizosphere regions by exerting changes in the quantity and variety of compounds lost through rhizodeposition and root senescence (Grayston et al. 1998, 2001). Hence, we surmise that the lack of differences in microbial community composition associated with root-C processing in rhizosphere versus non-rhizosphere soil in our study may have been because i) the root-C traced into both rhizosphere and non-rhizosphere soils was from the same source, i.e., cover crop rhizodeposits and ii) the

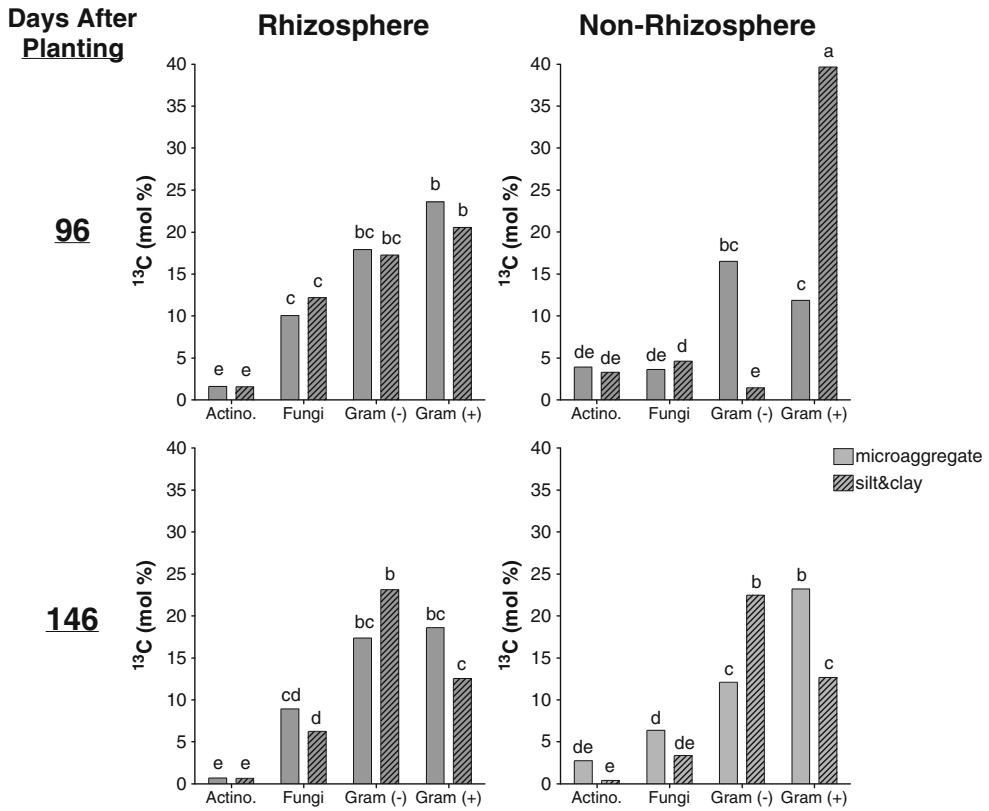

Fig. 5 Mole percentages of ^{13}C-PLFA (^{13}C mol%) associated with rhizosphere and non-rhizosphere soil in the low-input cropping system at 96 (*left panel*) and 146 days after planting (*right panel*). *Letters* above bars designate significant differences at the $p<0.05$ level ($n=3$)

origins of the rhizosphere community are similar to that of the non-rhizosphere community.

Cropping system effects on microbial community size and functioning within the rhizosphere and non-rhizosphere

Phospholipid fatty acid biomass extracted from rhizosphere and non-rhizosphere soils of the organic system were nearly double that of the low-input and conventional cropping systems and reflected the greater soil organic C stock in the organic system compared to the low-input and conventional systems, the latter of which were not different (Kong et al. 2011). This concurs with the results of Gunapala and Scow (1998) and Lundquist et al. (1999) who reported higher microbial biomass in organic than conventional cropping systems, which were located on soil types similar to those at the Russell Ranch experimental site. We tested the hypothesis that the first-time growth of hairy vetch in the conventional system would result in a smaller microbial community (e.g., less PLFA biomass) in the rhizosphere and slower rates of microbial C processing than in the low-input and organic systems. Although PLFA biomass in the conventional system was lower than in the organic system, the similarity in PLFA biomass and lack of differences in microbial C processing between the conventional and low-input systems at 96 DAP did not support our hypothesis. Perhaps measurement of PLFA biomass associated with only roots and rhizodeposition versus from the whole soil (root-C and other C inputs) reduced potential differences in PLFA biomass attributable to cropping system differences. Also, the similarity in the size of the microbial communities of the conventional and low-input systems may not come as a surprise since PLFA biomass is correlated to soil C levels (Powlson et al. 1987), which were similar between the conventional and low-input systems.

The similarities in the structure of the microbial communities actively processing root-C in the rhizosphere and non-rhizosphere among the conventional, low-input, and organic cropping systems (shown via ^{13}C mol% data) conflicted with the findings of Bossio et al. (1998) and Lundquist et al. (1999), whose PLFA analyses separated the microbial communities of conventional, low-input, and organic systems on soil types similar to this study. Nevertheless, our results are comparable to those of Buyer and Kaufman (1997) who, in a 15-year study on conventional maize-soybean and two low-input systems (i.e., animal manure and legumes), showed that total counts and microbial diversity were not significantly different for the three cropping systems. Because hairy vetch had never been grown in the conventional system of our study, which also received very different nutrient inputs compared to the low-input and organic systems, the lack of differences among the systems in rhizosphere microbial communities implied that the conventional system maintained a diverse microbial community that is capable of utilizing cover crop-C. This is in accordance with Hirsch et al. (2009), who compared soil microbial communities at the Rothamsted Highfield site under a mixed grass sward, arable rotation, and a 50-year old bare-fallow plot and found that the bare-fallow supported a species-rich, metabolically active bacterial community of similar diversity to that of the grass sward plot.

Interactions between the rhizosphere, soil microenvironments, and soil microorganisms

The most stable aggregates are typically encountered in the rhizosphere of plants, where plant exudates and microbial products provide the agents for aggregation (Kandeler and Murer 1993). In this study, ~52% of the rhizosphere was comprised of silt-and-clay, while ~38% of the soil was microaggregates, thereby suggesting that more silt-and-clay than microaggregate microenvironments are formed in the rhizosphere. We had expected that the soil microaggregate, which has been characterized by low predation pressure, relatively stable water potential, and low accessibility for exogenous toxic elements (Poly et al. 2001; Postma et al. 1989; Ranjard and Richaume 2001; Ranjard et al. 2000) would foster a microbial community with structure and functioning that enhanced C processing. Instead, we found i) more PLFA biomass in the silt-and-clay than in the microaggregates of the rhizosphere at the end of the cover crop growing season and ii) few differences in C_{new} incorporation into PLFA extracted from microaggregates compared to the silt-and-clay microenvironments. Although our hypothesis that the microbial community in the rhizosphere-microaggregate would lead to greater C processing than in the non-rhizosphere-microaggregate and the silt-and-clay fraction of either the rhizosphere or non-rhizosphere soils

was not corroborated, relative abundances of fungal biomarkers in the microaggregates were greater than in silt-and-clay in the rhizosphere but not in the non-rhizosphere. Tisdall and Oades (1982) suggested that microaggregates may be less restrictive for the growth of fungal hyphae, which may lead to the selection of fungi over bacteria in this microenvironment.

Although the C-utilizing microbial community associated with the silt-and-clay in the rhizosphere had a distinct PLFA profile from the microbial communities assimilating root-C in the microaggregates of the rhizosphere and in both the silt-and-clay and microaggregates of the non-rhizosphere (Fig. 4), our ^{13}C mol% results implied that the microbial communities utilizing root-C in the microaggregate and silt-and-clay microenvironments of the rhizosphere were similar. Therefore, we did not find that a particular microbial group dominated the processing of new root-C input in the rhizosphere or non-rhizosphere, when normalized for total C_{new} assimilation. The fact that these cropping systems are not constrained by nutrient limitations may have contributed to the similarities observed in C-utilization among the microbial communities in the microaggregates and silt-and-clay of the rhizosphere and non-rhizosphere.

Conclusions

Across the conventional, low-input, and organic cropping systems, PLFA biomass and root-C incorporated into microbial groups were greater in rhizosphere than non-rhizosphere soil. Therefore, our hypothesis that more C is processed within soil microbial communities in the rhizosphere than non-rhizosphere soil was supported. Together with findings from a companion study, which showed more root-C than residue-C is assimilated into PLFA after the cover crop growing season (Kong et al. 2011), the current study suggests that rhizodeposition might play a major role in the greater contribution of root-C than residue-C to SOM stabilization. Our hypothesis that the first-time growth of vetch in the conventional system would result in less PLFA biomass, a different microbial community structure, and slower rates of microbial C processing than in the low-input and organic systems, however, was not corroborated. The microbial communities utilizing root-C in the rhizosphere and non-rhizosphere in all three systems were structurally and functionally similar, with no particular microbial group dominating new root-C processing in the rhizosphere or non-rhizosphere; thereby, suggesting that the cropping systems maintained diverse microbial communities that are capable of utilizing the cover crop-C despite receiving different long-term nutrient inputs. Our hypothesis that the microbial community in the rhizosphere-microaggregate would lead to greater C processing than in the non-rhizosphere-microaggregate and the silt-and-clay of either rhizosphere or non-rhizosphere soils was also not supported. Lastly, results from this study imply that microbial communities responsible for C processing are sensitive to their microenvironment (i.e., microaggregates versus silt-and-clay), but differences in long-term crop management did not lead to differences in the capacity of the microbial communities to utilize cover crop-derived C.

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