Microbial community composition and carbon cycling within soil microenvironments of conventional, low-input, and organic cropping systems

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A B S T R A C T
This study coupled stable isotope probing with phospholipid fatty acid analysis (\(^{13}\)C-PLFA) to describe the role of microbial community composition in the short-term processing (i.e., C incorporation into microbial biomass and/or deposition or respiration of C) of root- versus residue-C and, ultimately, in long-term C sequestration in 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 (Zea mays – Lycopersicum esculentum) cropping systems. During the maize growing season, we traced \(^{13}\)C-labeled hairy vetch (Vicia dasycarpa) roots and residues into PLFAs extracted from soil microaggregates (53–250 \(\mu\)m) and silt-and-clay (<53 \(\mu\)m) particles. Total PLFA biomass was greatest in the organic (41.4 nmol g\(^{-1}\) soil) and similar between the conventional and low-input systems (31.0 and 30.1 nmol g\(^{-1}\) soil, respectively), with Gram-positive bacterial PLFA dominating the microbial communities in all systems. Although total PLFA-C derived from roots was over four times greater than from residues, relative distributions (mol\%) of root- and residue-derived C into the microbial communities were not different among the three cropping systems. Additionally, neither the PLFA profiles nor the amount of root- and residue-C incorporation into the PLFAs of the microaggregates were consistently different when compared with the silt-and-clay particles. More fungal PLFA-C was measured, however, in microaggregates compared with silt-and-clay. The lack of differences between the mol\% within the microbial communities of the cropping systems and between the PLFA-C in the microaggregates and the silt-and-clay may have been due to (i) insufficient differences in quality between roots and residues and/or (ii) the high N availability in these N-fertilized cropping systems that augmented the abilities of the microbial communities to process a wide range of substrate qualities. The main implications of this study are that (i) the greater short-term microbial processing of root- than residue-C can be a mechanistic explanation for the higher relative retention of root- over residue-C, but microbial community composition did not influence long-term C sequestration trends in the three cropping systems and (ii) in spite of the similarity between the microbial community profiles of the microaggregates and the silt-and-clay, more C was processed in the microaggregates by fungi, suggesting that the microaggregate is a relatively unique microenvironment for fungal activity.

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1. Introduction

Concerns about long-term agricultural sustainability and the high environmental costs of conventional cropping practices have made it imperative that soil and crop management practices that enhance carbon (C) sequestration, improve soil quality, and mitigate global greenhouse gas emissions be developed (Pimentel et al., 2005; Robertson and Paul, 1998). Alternative crop management practices, such as cover cropping, additions of composted manure, and reducing or eliminating synthetic fertilizer use, have emerged as integrated and ecologically sound approaches to increasing soil organic matter (SOM) levels (Poudel et al., 2001). A better mechanistic understanding of the nutrient cycling under alternative cropping practices is necessary in order to promote their benefits to soil and environmental health.

In a recent study comparing the effects of long-term conventional and alternative cropping systems on SOM stabilization (Kong and Six, 2010), higher relative retention of root-C versus residue-C...
was shown in organic (annual composted manure and cover crop additions) compared with conventional (annual synthetic fertilizer applications) and low-input (synthetic fertilizer and cover crop applied in alternating years) irrigated, maize-tomato systems (Zea mays-Lycopersicum esculentum) (Table 1). This trend mirrored the substantial differences in soil C stock among the systems (i.e., organic > low-input = conventional systems) after long-term (10 + years) implementation of the respective crop management practices (see Table 2 in Kong and Six, 2010). The rate of C sequestration in the organic system was ~14 times faster than the rates of C sequestration in both the conventional and low-input systems. Yet, the greater C sequestration rate in the organic system was not proportional to its relative rate of C input, which was 1.7 times more than in the conventional system (Kong et al., 2005). The data suggested that preferential stabilization of belowground cover crop C inputs in the organic system was integral to its disproportionately higher total soil C sequestration (Kong and Six, 2010).

It has been shown that agricultural management practices (e.g., tillage, cropping rotation, and irrigation) strongly influence the soil environment and, consequently, the size, composition, and function of the microbial community in soils (Beare et al., 1992; Bossio et al., 1998; Buyer and Kaufman, 1997; Frey et al., 1999; Lundquist et al., 1999). In turn, microorganisms strongly influence a wide range of soil processes, properties, and functions in agricultural and natural ecosystems, such as the cycling of organic C compounds and the formation and stabilization of soil structure (Kennedy and Papendick, 1995; Oades, 1983). Nitrogen addition and availability often stimulate mineralization of readily available plant-C (Fog, 1988; Henriksen and Breland, 1999; Recous et al., 1995); therefore, higher rates of organic matter decomposition by soil microorganisms can be expected in cropping systems with more available N. Because the cropping systems in the study by Kong and Six (2010) did not differ in soil type, crop species, or amount of cover crop residue added, it was postulated that the greater total soil C content and the greater stabilization of root-C versus residue-C in the organic system was the result of more available soil N that stimulated C processing (i.e., C utilization by microorganisms that includes C incorporation into the microbial biomass and/or deposition or respiration of C) within the microbial community and, therefore, increased the stabilization of microbial-derived C in the soil compared to within the conventional and low-input systems.

Changes in soil structural properties induced by agricultural management practices impact soil aggregation (Elliott, 1986; Tisdall and Oades, 1982), which governs the chemical and physical heterogeneity of soil properties, and, consequently, the distribution of microorganisms and their activity among aggregates of different sizes (Gupta and Germida, 1988; Kuikman et al., 1990; Schutter and Dick, 2002). It is still unclear, however, how differences in microbial communities within different microenvironments in the soil matrix affect soil C processing rates at the cropping system-scale. For example, the soil microaggregate is an operationally defined microenvironment for microorganisms, characterized by low predation pressure, relatively stable water potential, low O2 availability, and low accessibility for exogenous toxic elements (Poly et al., 2001; Postma et al., 1989; Ranjard and Richaume, 2001; Ranjard et al., 2000). Because the microaggregate is an ideal microenvironment for microbial growth and is potentially more important in long-term C sequestration than other aggregate fractions (Six et al., 2000; Skjemstad et al., 1990), it is essential to gain a better understanding of the relationship between short-term microbial-mediated C cycling within microaggregates and long-term SOC sequestration in the whole soil.

Recently, characterization of the chemical structure and isotopic composition of soil organic C has revealed a low stability of litter-derived lignin in soil (Gleixner et al., 2002; Rasse et al., 2006). Recent studies have also highlighted the importance of the long-term stabilization of N-containing compounds (proteins, amino acids, peptides, chitin) and polysaccharides to soil organic C stabilization in arable soils (Kiem and Kögel-Knabner, 2003). These findings imply a preservation of microbial-derived C and indicate the greater importance of microbial processing of organic matter over intrinsic biochemical recalcitrance for the long-term stabilization of C (Deneo et al., 2009; Gentile et al., 2010; Kögel-Knabner, 2002). Although a proportion of organic matter-C is respired during microbial processing, we maintain that more microbial activity is a prerequisite for greater C sequestration, in absolute terms (i.e., increased C respiration is not necessarily contradictory to increased C stabilization). To investigate the relationship between the activity and structure of the soil microbial community and short-term root-versus residue-C processing in conventional, low-input, and organic maize-tomato cropping systems, we 13C-labeled hairy vetch (Vicia dasyarpa) roots and residues and traced 13C into the phospholipid fatty acids (PLFAs) of microorganisms within soil microaggregates and silt-and-clay particles of the three cropping systems. Our overall objective was to link differences in long-term soil C sequestration among the cropping systems to differences in

### Table 1

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<tr>
<th>Days after incorporation</th>
<th>Root</th>
<th>Cropping system</th>
<th>Conventional</th>
<th>Low-input</th>
<th>Organic</th>
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<td>kg Cnew ha⁻¹</td>
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<td>148 (ab)</td>
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<td>194 (c)</td>
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<td>69</td>
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<td>196 (bc)</td>
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<tr>
<td>100</td>
<td>278 (b)</td>
<td>354 (ab)</td>
<td>na</td>
<td>103 (cd)</td>
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<tr>
<td>134</td>
<td>154 (c)</td>
<td>283 (b)</td>
<td>401 (a)</td>
<td>109 (cd)</td>
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<th>Days after incorporation</th>
<th>Residue</th>
<th>Cropping system</th>
<th>Conventional</th>
<th>Low-input</th>
<th>Organic</th>
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microbial community structure and short-term C processing. The following specific hypotheses were tested: (i) microbial communities process root-C more rapidly than residue-C, thereby, leading to more C sequestration in organic than conventional and low-input cropping systems and (ii) microbial processing of both root- and residue-C are greater in the microaggregate than the silt-and-clay microenvironment.

2. Materials and methods

2.1. Experimental site and design

The field study took place during the 2006 maize growing season on 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). Located in a Mediterranean climate region (e.g., wet winters and hot, dry summers), the Russell Ranch site is comprised of two soil types: i) Yolo silty loam (fine-silty, mixed, nonacid, thermic Typic Xerorthent) and ii) Rincon silty clay loam (fine, montmorillonitic, thermic Mollic Haploxeralf). This field study compared the conventional, low-input, and organic maize-tomato cropping systems (Table 2), which were arranged in a completely randomized design, with three 0.4 ha replicates that received furrow irrigation and have been under standard tillage since 1993.

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 was incorporated into the organic cropping system at a rate of 373 kg N ha⁻¹. Maize was direct-seeded into the same cropping system replicate (henceforth, referred to as the system plot, composited, and then subsampled for baseline soil content, and stored at 5 °C). The mass of the extracted roots was used to extrapolate the root biomass for the microplot to a depth of 15 cm. Hairy vetch root biomass was estimated to be 153 g m⁻². We acknowledge that our root estimation procedures, which probably excluded very fine roots and overlooked dynamic C contributions from root exudates, likely underestimated total-root-derived C inputs.

To incorporate the hairy vetch, aboveground biomass from both microplots were mowed and promptly weighed and subsampled for elemental and isotopic C concentrations, as well as to determine dry matter content. Standing aboveground biomass averaged 554 g m⁻² and did not differ (P > 0.05). The concentration of 750 ppm CO₂ (99 atom%) was monitored over the experimental plots at sunset after each labeling event using a portable infrared gas analyzer (Qubit CO₂ Analyzer, Model S-151, Qubit Systems, Kingston, Ontario, Canada) and the chamber CO₂ per labeled microplot. The portable ¹³C-labeling chambers consisted of a vinyl sheet (TAP Plastics, Sacramento, CA) fitted around a polyvinyl chloride frame (height adjustable), with excess vinyl that was fixed against the contours of the soil surface to serve as a seal around the edges of the frame. During the ¹³C-labeling procedure, ¹³C-CO₂ was injected into the chambers to gain a CO₂ concentration of 750 ppm. The CO₂ concentration was monitored using a portable infrared gas analyzer (Qubit CO₂ Analyzer, Model S-151, Qubit Systems, Kingston, Ontario, Canada) and the chamber was removed when the CO₂ level in the chamber dropped below 250 ppm (usually after 30–45 min). The ¹³C-labeling events took place between the hours of 1100 and 1300. To maximize plant uptake of the ¹³CO₂, the labeling chambers were placed once more over the experimental plots at sunset after each labeling event (~1700 h) to capture overnight ¹³CO₂ respiration, and then removed the following morning (~800 h) after CO₂ levels in the chamber dropped below 250 ppm. Air temperatures in the chambers during ¹³C-labeling ranged from 25 °C to 30 °C.

2.2. ¹³C pulse-labeling of hairy vetch

Hairy vetch (V. dasycarpa Ten.) seeds were broadcast-sown within each of the cropping treatment replicates at the end of October 2005 (i.e., the start of the winter cover crop growing season). Two microplots (1.0 × 1.0 m) were established in each treatment replicate, shortly after hairy vetch germination. At five weekly events between March 26 and April 26, 2006, one of the two microplots was enclosed with a portable chamber and pulse-labeled with ¹³C-CO₂ (99 atom%), for a cumulative total of 6.5 l ¹³C-CO₂ per labeled microplot. The portable ¹³C-labeling chambers consisted of a vinyl sheet (TAP Plastics, Sacramento, CA) fitted around a polyvinyl chloride frame (height adjustable), with excess vinyl that was fixed against the contours of the soil surface to serve as a seal around the edges of the frame. During the ¹³C-labeling procedure, ¹³C-CO₂ was injected into the chambers to gain a CO₂ concentration of 750 ppm. The CO₂ concentration was monitored using a portable infrared gas analyzer (Qubit CO₂ Analyzer, Model S-151, Qubit Systems, Kingston, Ontario, Canada) and the chamber was removed when the CO₂ level in the chamber dropped below 250 ppm (usually after 30–45 min). The ¹³C-labeling events took place between the hours of 1100 and 1300. To maximize plant uptake of the ¹³CO₂, the labeling chambers were placed once more over the experimental plots at sunset after each labeling event (~1700 h) to capture overnight ¹³CO₂ respiration, and then removed the following morning (~800 h) after CO₂ levels in the chamber dropped below 250 ppm. Air temperatures in the chambers during ¹³C-labeling ranged from 25 °C to 30 °C.

2.3. Estimating biomass and ¹³C contributions of roots and residues

In the final week of April, immediately before hairy vetch incorporation, soil cores (4 cm diameter; 0–15 cm) were collected from all microplots to determine belowground standing root biomass and the δ¹³C values of both labeled and unlabeled roots. Three field moist soil samples per microplot were collected and large visible roots were immediately removed from these samples. Approximately 100 g soil was subsampled from the soil core and suspended in ~200 mL deionized water. The slurry was stirred by hand and the roots that separated from the soil were removed and placed in petri dishes on ice. Visual criteria (e.g., color and elasticity) were used to distinguish hairy vetch roots from roots of other plants. The remainder of the core (~250 g field moist soil) was subsampled and processed for roots in the same manner as above. Roots were rinsed under a gentle stream of deionized water to remove soil and then dried at 50 °C. The mass of the extracted roots was used to extrapolate the root biomass for the microplot to a depth of 15 cm. Hairy vetch root biomass was estimated to be 153 g m⁻². We acknowledge that our root estimation procedures, which probably excluded very fine roots and overlooked dynamic C contributions from root exudates, likely underestimated total-root-derived C inputs.

To incorporate the hairy vetch, aboveground biomass from both microplots were mowed and promptly weighed and subsampled for elemental and isotopic C concentrations, as well as to determine dry matter content. Standing aboveground biomass averaged 554 g m⁻² and did not differ (P > 0.05). The concentration of 750 ppm CO₂ (99 atom%) was monitored over the experimental plots at sunset after each labeling event using a portable infrared gas analyzer (Qubit CO₂ Analyzer, Model S-151, Qubit Systems, Kingston, Ontario, Canada) and the chamber CO₂ per labeled microplot. The portable ¹³C-labeling chambers consisted of a vinyl sheet (TAP Plastics, Sacramento, CA) fitted around a polyvinyl chloride frame (height adjustable), with excess vinyl that was fixed against the contours of the soil surface to serve as a seal around the edges of the frame. During the ¹³C-labeling procedure, ¹³C-CO₂ was injected into the chambers to gain a CO₂ concentration of 750 ppm. The CO₂ concentration was monitored using a portable infrared gas analyzer (Qubit CO₂ Analyzer, Model S-151, Qubit Systems, Kingston, Ontario, Canada) and the chamber was removed when the CO₂ level in the chamber dropped below 250 ppm (usually after 30–45 min). The ¹³C-labeling events took place between the hours of 1100 and 1300. To maximize plant uptake of the ¹³CO₂, the labeling chambers were placed once more over the experimental plots at sunset after each labeling event (~1700 h) to capture overnight ¹³CO₂ respiration, and then removed the following morning (~800 h) after CO₂ levels in the chamber dropped below 250 ppm. Air temperatures in the chambers during ¹³C-labeling ranged from 25 °C to 30 °C.
We acknowledge that, because we did not collect soil samples immediately before the termination of the cover crop growing season, root exudates and other C inputs from rhizodeposition cannot be partitioned from C derived from root decomposition during the maize growing season in our estimates of SOM derived from root-C. In this study, root-C estimates include both rhizodeposition and root decomposition, while residue-C estimates represent residue decomposition.

2.5. Soil microenvironment isolation

From each soil sample, 30 g subsamples were physically fractionated into two soil microenvironments, operationally defined as microaggregates (53–250 μm) and the silt-and-clay fraction (<53 μm). Frozen soil samples were thawed for 20 min and submerged in deionized water at room temperature for 5 min to slake the soil. Soil microenvironments were obtained using a microaggregate isolator (Six et al., 2000) in which the slaked soil was immersed in deionized water on top of a 250 μm mesh screen and gently shaken with 50 stainless steel beads (4 mm diameter) until only >250 μm particulate organic matter 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. To separate water-stable microaggregates from silt-and-clay particles, the material on the 53 μm sieve was manually sieved over a 2 min period by moving the sieve 50 times in an up-and-down motion (Elliott, 1986). Microaggregate, silt-and-clay, and the >250 μm particulate organic matter fractions were collected as soil suspensions and centrifuged at 5000 rpm for 15 min at 4 °C (Sorvall RC-5C Plus Superspeed centrifuge, Thermo Scientific). The supernatant was discarded and the remaining material was lyophilized and stored at −20 °C until further analysis. To determine whether microbial biomass/PLFA may have been transferred from the larger aggregate fractions to the smaller microenvironments during each successive wet-sieving, we measured total organic C (TOC) concentrations of the supernatant from each fraction. Negligible amounts of TOC (~10 mg L−1) were washed from the >250 μm material to the microaggregates and then to the silt-and-clay. For this study, only data pertaining to the microaggregate and silt-and-clay will be discussed as these fractions consisted predominantly of mineral soil, whereas the >250 μm material was largely comprised of plant material and sand.

The distributions of soil microenvironments were similar between the cropping systems and did not change from the time-zero to the harvest sampling events. At 29 DAI, 58.0–64.7% of the soil mass was comprised of silt-and-clay fraction (<53 μm), which was the greatest SOM fraction by weight across the systems. Meanwhile, the microaggregate (53–250 μm) was the greatest SOM fraction by weight across the systems.

2.6. PLFA extraction, quantification, and 13C analysis

Phospholipid fatty acids (PLFAs) were extracted from the microaggregates and silt-and-clay according to the procedure of Bossio and Scow (1998). Total lipids were extracted from 4 g of lyophilized sample using a potassium phosphate buffer, chloroform, and methanol buffer. Phospholipids were fractionated from neutral and glycolipids on a silicic acid column (Supelco, Inc., Bellefonte, PA). After mild alkaline methanolysis to form fatty acid methyl esters (FAMES), FAMES were analyzed using a Thermo gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) system, which consisted of a Trace GC Ultra gas chromatograph (Thermo Electron Corp., Milan, Italy) coupled to a Delta Plus Advantage isotope ratio mass spectrometer through a GC/C-III interface (Thermo Electron Corp., Bremen, Germany). With He as the carrier gas, FAMES were injected in splitless mode and resolved in a J&W DB-5 column (30 m × 0.25 mm ID, 0.25 μm film thickness) with an initial temperature of 60 °C for 0.1 min, ramped to 150 °C at 10 °C min−1 and held for 2 min, ramped to 220 °C at 3 °C min−1, ramped to 280 °C at 2 °C min−1 and held for 5 min, and quantitatively converted to CO2 in an oxidation reactor at 950 °C. Carbon dioxide of known isotopic composition was injected at the beginning and end of each run. 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.). Quantification of chromatographic peaks was accomplished by comparing peaks to an internal standard [nonadecanoic FAME (19:0)]. Data were expressed as absolute 12C/PLFA biomass (nmol g−1 dry soil) and mole percentages (mol%), which were calculated as the area of each PLFA peak relative to the summed area of all PLFA peaks, after adjusting for the number of C atoms per mole of PLFA.

Enrichment values of individual FAMES were corrected for the C atom introduced during the addition of the methyl group during transesterification by mass balance:

$$\delta_{13C}^{PLFA} = \frac{\left(\frac{N_{PLFA} + 1}{N_{PLFA}}\right)\delta_{13C}^{PLFA}_{FAME} - \delta_{13C}^{PLFA}_{MeOH}}{N_{PLFA}}$$

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

Standard fatty acid/PLFA nomenclature (A:BoC) 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, respectively. Notations ‘Me’, ‘cy’, ‘i’, and ‘t’ 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 the 40 FAME peaks that were detected, on average in each sample, 19 FAMES that were present in abundances greater than 1 mol% for both 13C/PLFA and total PLFA-C (~52% of the total PLFA concentration) and in sufficient quantity to obtain accurate $\delta^{13C}$ values were used in the PLFA analyses. Fatty acids 18:1ω9 and 18:3ω3 were not resolved and eluted as one peak (designated 18:1ω9), as did 18:1ω9t and 18:1ω7c (designated 18:1ω7t). Phospholipid fatty acid biomarkers were assigned to six microbial categories: 15:0, 17:0, 16:0, 17:0, 15:0, 17:0 for Gram-positive bacteria (Zelles, 1999); 18:1ω7, 17:0, cy17:0 for Gram-negative bacteria (Zelles, 1999); 18:2ω6 and 18:1ω9 were used as indicators of fungi (Frostegård and Bååth, 1996; Zelles, 1997), while 10Me16:0 and 10Me18:0 were used to indicate actinomycetes (Zelles, 1999). Phospholipid fatty acids not assigned as biomarkers (12Me16:0, 16:1 2–OH, 16:0, 18:0, 16:1ω5, 16:1ω9c, 16:1ω9t) were included in total PLFA yields. Fungal/bacterial ratios were calculated as fungal group PLFA yield (nmol g−1 soil) divided by the sum of Gram-positive and Gram-negative bacterial PLFA groups (Zelles, 1999).

The proportion (f) of PLFA-C in the soil microenvironments derived from either 13C-labeled residue or root biomass was calculated as follows:

$$f = \frac{\delta^{13C}_{PLFA} - \delta^{13C}_{natural abundance PLFA}}{\delta^{13C}_{natural abundance PLFA}}$$

where $\delta^{13C}_{PLFA}$ is the $\delta^{13C}$ for the PLFA of interest, $\delta^{13C}_{natural abundance PLFA}$ is the background $\delta^{13C}$ for residue or root biomass, and $\delta^{13C}_{natural abundance PLFA}$ is the $\delta^{13C}$ of the equivalent PLFA taken at the time-zero sampling event. Total PLFA-
C concentrations in the soil microenvironments were multiplied by \( f \) to obtain PLFA-\( C_{\text{new}} \), the concentration of PLFA-C derived from either \( ^{13}\text{C} \)-labeled residue or root biomass.

2.7. Data and statistical analyses

Within one sampling event, PLFA-C and \( C_{\text{new}} \) concentrations as well as mole percentages among the cropping systems and soil microenvironments were analyzed as a split-plot, completely randomized design using the PROC MIXED procedure of the Statistical Analysis System (SAS; SAS Institute, 2002), with cropping system as the main plot, the source of \( ^{13}\text{C} \) (‘\( C \) source’; i.e., root or residue) as the sub-plot, and plot was included as a random factor. To compare differences in PLFA-C, PLFA-\( C_{\text{new}} \) concentrations and mole percentages among the soil sampling events, repeated measures analyses were performed using the PROC MIXED procedure. These data were analyzed as a split-plot, completely randomized design with cropping system as the main plot, \( C \) source as the sub-plot, and plot as a random factor. A standard variance components covariance structure was specified with the TYPE= VC option in the model statement. 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.

3. Results

3.1. Biomass and biomarker distribution

Across the sampling events and cropping systems, total PLFA biomass (nmol PLFA g\(^{-1}\) dry soil) was similar between the root and residue microplots (36.6 and 35.5 nmol PLFA g\(^{-1}\) soil, respectively). Total PLFA biomass, averaged for the three cropping systems, was lower at 134 DAI (31.4 nmol PLFA g\(^{-1}\) soil) than the other sampling events; yet, averaged across the sampling events, higher PLFA biomass was measured in the organic (43.9 nmol PLFA g\(^{-1}\) soil) compared with the low-input (31.4 nmol PLFA g\(^{-1}\) soil) and conventional systems (32.8 nmol PLFA g\(^{-1}\) soil). Additionally, greater PLFA biomass was associated with the silt-and-clay (38.1 nmol PLFA g\(^{-1}\) soil) than the microaggregate (34.0 nmol PLFA g\(^{-1}\) soil) fraction, across all sampling events and cropping systems.

Because the overall trends in the relative abundances of the microbial biomarkers (nmol biomarker g\(^{-1}\) dry soil/total nmol biomarker g\(^{-1}\) dry soil) were similar among the sampling events, Fig. 1 shows the distribution of biomarkers only at 134 DAI. Relative abundances of the microbial biomarkers at 134 DAI decreased in all cropping systems in the following order: Gram-positive (25.5%) > Gram-negative (17.6%) > fungi (8.5%) > actinomycetes (6.3%) (\( p < 0.05 \); Fig. 1). At 134 DAI, higher percentages of fungal and Gram-positive bacterial biomarkers were found in the microaggregates (9.2 and 27.2%, respectively) than in the silt-and-clay (6.5 and 25.7%, respectively), but proportions of PLFA biomarkers for Gram-negative and actinomycetes were not different between the silt-and-clay and microaggregates (Fig. 1). Across all sampling events and cropping systems, a \( C \) source \( \times \) biomarker effect showed that only the root-derived fungal biomarkers (8.8%) were greater than residue-derived fungal biomass (8.2%), while the other \( C \) source-biomarker pairs were not different (\( p < 0.05 \); data not shown).

At 134 DAI, the fungal:bacterial ratios across the cropping systems and microenvironments ranged from 0.12 to 0.24 and were, on average, significantly lower than the ratios at 29 DAI (0.16–0.26; Table 3). Fungal:bacterial ratios at 29 and 134 DAI were similar among the three cropping systems (Table 3). However, fungal:bacterial ratios for microaggregates (0.23) were higher than ratios for the silt-and-clay fractions (0.16) averaged across systems and sampling events.

3.2. Root- and residue-derived PLFA-C

Across the three cropping systems, trends in \( ^{13}\text{C} \)-PLFA concentrations at 69, 100, and 134 DAI were similar; hence, we report only

![Fig. 1. Phospholipid fatty acid (PLFA) biomass distribution of microbial biomarkers (nmol g\(^{-1}\) dry soil/total nmol g\(^{-1}\) dry soil) at the harvest sampling event (134 days after vetch incorporation). Letters above bars indicate differences between microbial groups but within cropping systems and soil microenvironments (\( n = 3; p < 0.05 \)).](image-url)
data from 29 to 134 DAI (Figs. 2, 3, and 4). Recoveries of $^{13}$C-PLFA ranged between 0.01 and 19 nmol PLFA-C new g$^{-1}$ soil, which is comparable to the 12.4 nmol C g$^{-1}$ soil recovery reported in Moore-Kucera and Dick (2008). At 29 DAI, PLFA-C derived from root-C (2.53 nmol PLFA-C new g$^{-1}$ soil) was approximately 2.8 times higher than residue-derived PLFA-C. By 134 DAI, total $^{13}$C-PLFA concentrations had decreased nearly 50% from 29 DAI, but root-derived PLFA-C new (2.01 nmol PLFA-C new g$^{-1}$ soil) remained greater than PLFA-C new derived from residue-C (0.34 nmol PLFA-C new g$^{-1}$ soil). Across the cropping systems, $^{13}$C-PLFA concentrations were higher in the silt-and-clay (2.37 nmol PLFA-C new g$^{-1}$ soil) than the microaggregates (1.85 nmol PLFA-C new g$^{-1}$ soil) at 29 DAI, but not at 134 DAI. Among the three cropping systems, the highest concentrations of $^{13}$C-PLFA were found in the conventional system, specifically in PLFAs, a15:0, 18:1u7, cy17:0, i15:0, and 16:0. In all cropping systems, at both 29 and 134 DAI, the highest concentrations of either root- or residue-derived PLFA-C were found in 18:1u7 and 16:0 (4.35 and 4.72 nmol PLFA-C new g$^{-1}$ soil, respectively).

A significant soil microenvironment × PLFA effect showed that, with the exception of the root-derived PLFA of the conventional system at 29 DAI (Fig. 2), both root- and residue-derived fungal $^{13}$C-PLFA were greater in the microaggregate than the silt-and-clay of the three cropping systems. At 29 DAI, PLFAs a15:0, 18:1u7, and

<table>
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<th>Days after incorporation</th>
<th>Soil microenvironment</th>
<th>Root</th>
<th>Residue</th>
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<td>Cropping systems</td>
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<td>Low-input</td>
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<td>(nmol g$^{-1}$ soil)/(nmol g$^{-1}$ soil)</td>
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<td>Silt-and-clay</td>
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<td>134</td>
<td>Microaggregate</td>
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<td></td>
<td>Silt-and-clay</td>
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Table 3
Ratios of fungal:bacterial phospholipid fatty acid (PLFA) biomass (nmol g$^{-1}$ soil) associated with the microaggregate (53–250 μm) and silt-and-clay (<53 μm) microenvironments in the conventional, low-input, and organic cropping systems, at 29 and 134 days after vetch incorporation (DAI). Values in bold indicate significantly higher ratios for a soil microenvironment effect, while * indicates significantly higher fungal:bacterial PLFA biomass ratios at 29 DAI. Statistical significance was determined at $p < 0.05$. 

Fig. 2. Concentration of $^{13}$C-PLFA in the soil microenvironments (microaggregates and silt-and-clay fraction) of the conventional cropping system: nmol g$^{-1}$ dry soil (A) derived from roots 29 days after vetch incorporation (DAI), (B) derived from residues at 29 DAI, (C) derived from roots at 134 DAI, and (D) derived from residues at 134 DAI. Letters above bars indicate differences across PLFAs but within a C source and sampling event ($n = 3$; $p < 0.05$).
16:0 in the silt-and-clay of the low-input and organic systems were more $^{13}$C-enriched than the microaggregates (Figs. 3 and 4). Actinomycete biomarkers, 10Me16:0 and 10Me18:0, showed higher $^{13}$C-PLFA concentrations in the silt-and-clay than the microaggregates only at 29 DAI of the low-input system, but were not different in any of the other cropping systems. Differences between root-C distribution across PLFAs of the silt-and-clay versus microaggregates were most pronounced for the organic system at 134 DAI, where we found greater $^{13}$C yields in 18 of 19 PLFAs in the microaggregates compared to the silt-and-clay ($p < 0.1$; Fig. 4).

### 3.3. Microbial community preferences of root- versus residue-derived C

Whereas root contributions to total $^{13}$C-PLFA concentrations were higher than those from residue-C, relative distribution of root- and residue-derived $^{13}$C-PLFA (mol%), which were normalized for the amount of total $^{13}$C in each sample, were not different at any of the sampling events or among the cropping systems. Hence, only mol% data for the organic system at 134 DAI is shown in Fig. 5. The communities actively utilizing residue- and root-C were dominated by Gram-positive and Gram-negative bacteria (20 mol%), while actinomycetes were the least active in either root- or residue-C assimilation (5 mol%). Nevertheless, the distribution of microbial groups (e.g., Gram-positive, Gram-negative, etc.) utilizing either root-C or residue-C were not different. Additionally, relative distribution of root- and residue-derived C was not different between the communities within the microaggregates (14.7 mol%) and the silt-and-clay (13.5 mol%).

### 4. Discussion

#### 4.1. Long-term crop management influences on microbial community structure and C processing

In Kong and Six (2010), we suggested that preferential stabilization of root-C inputs over residue-C in the organic system could explain the disproportionately higher C sequestration observed for the organic compared with the conventional and low-input systems. In this study, we investigated whether short-term C processing by the microbial community was directly responsible for the relationship between root-C stabilization and long-term soil C sequestration in the three systems. We found that total PLFA biomass was higher in the organic compared with the low-input and conventional systems and that total PLFA biomass reflected the trends in long-term soil C stocks (organic > conventional = low-input). In a field site near Russell Ranch, Gunapala and Scow (1998) and Lundquist et al. (1999) have also shown higher microbial biomass in organic compared to conventional systems, which were on soil types similar to those at Russell Ranch.

Despite differences in total PLFA biomass, cropping system management (e.g., different N-fertilizer quality and application rates) did not influence the relative abundances of biomarkers (Fig. 1), fungal:bacterial biomass ratios (Table 3), or microbial
community $^{13}$C processing among the systems. Because this study marked the first instance in which hairy vetch was grown in the conventional cropping system, we were surprised to find that microbial processing of hairy vetch-C in the conventional did not differ from the low-input and organic systems, which regularly received cover crop additions. This suggested that C processing rates within the microbial populations in the cropping systems were at capacity and the addition of more C did not stimulate C processing by the microbial community. Hence, our hypothesis that the microbial community in the organic crop management would enhance hairy vetch root-C versus residue-C processing compared with the microbial communities of the low-input and conventional systems was not supported.

The lack of differences among the $^{13}$C processing microbial communities (and total C processing communities; data not shown) of the conventional, low-input, and organic systems in our study conflicted with the findings of Bossio et al. (1998) and Lundquist et al. (1999), whose analyses of PLFA clearly differentiated the microbial communities of the low-input, organic, and conventional systems from agricultural experiments very similar to this one. Had we collected soil samples during the cover crop growing season, we might have captured some differences between the microbial communities in the systems as the communities were assimilating a steady flow of $^{13}$C from root exudates and sloughed cells during this period. Vetch residue incorporation, ample additions of organic and mineral N inputs, and cultivation in all three of the systems might have diminished differences in factors that determine the microbial community structure (e.g., soil moisture, pH levels, N availability, etc.).

4.2. Microbial community processing of root- versus residue-derived C

Plant quality can affect microbial community composition and function during residue decomposition (Bending et al., 2002). It is well established that root and shoot residues may decompose at different rates and persist in microbial and soil C pools for time periods that vary considerably (Gale et al., 2000; Puget and Drinkwater, 2001; Rasse et al., 2005). Williams et al. (2006) showed that the amount of PLFA-C derived from ryegrass root-C (28%) was greater than the contribution from ryegrass straw-C (~5%). Although the cropping systems received over five times more residue-C input than root-C input, our data concur with the findings of Williams et al. (2006) in that $^{13}$C-PLFA derived from root-C was over four times higher than $^{13}$C-PLFA derived from residue-C (across the season and cropping systems). The greater contribution of root-C than residue-C to the PLFAs might be due to root exudation and the sloughing of root cells during the cover crop growing season, which our procedures probably underestimated. However, the similarity of the relative distribution of $^{13}$C into PLFAs associated with both root- and residue-C implied that, when corrected for the amount of C incorporation, the microbial community utilizing either C source was not different (Fig. 5a–d). Similarly, Moore-Kucera and Dick (2008) did not find a difference
between the compositions of soil microbial communities associated with litter versus root decomposition in a forest system. We suggest that the lack of differences in overall $^{13}$C mol% between root-derived and residue-derived PLFA-C may have been due to: (i) an insufficient difference in the quality of hairy vetch roots (C:N ratio = 15.7) versus residues (C:N ratio = 13.5) as differences in the quality of plant materials strongly influence their decomposition (Heal et al., 1997) and/or (ii) the high N availability in such N-fertilized cropping systems (>220 kg N ha$^{-1}$) removing N constraints on microbial metabolism, thereby, augmenting microbial decomposition of a variety of substrate types (Bragazza et al., 2006).

The relatively low fungal:bacterial $^{13}$C-PLFA ratios (~0.23; data not shown) at both 29 and 134 DAI suggested that bacteria dominated the decomposition processes throughout the season. This does not concur with soil microbial community decomposition models where bacteria (first Gram-negative, then Gram-positive) dominate the early stages of decomposition and fungi dominate at later stages (Coleman et al., 1983; Moore and de Ruiter, 1991). However, the dominance of bacterial PLFA in the three systems might have resulted from the level of N in each of these cropping systems (>220 kg N ha$^{-1}$) removing N constraints on microbial metabolism, thereby, augmenting microbial decomposition of a variety of substrate types (Bragazza et al., 2006).

4.3. Soil aggregates as microenvironments and selective components of microbial distribution and activity

The preference of a microbial group for a specific location in the soil matrix to grow and utilize substrates may be related to the accessibility to substrates but also, and perhaps more importantly, to the unique physicochemical conditions of that soil habitat. It has been shown that the size, stability, and chemical properties of soil aggregates influence the spatial distribution, functioning, and diversity of bacterial populations at the microscale (Gupta and Geremia, 1988; Mumme and Stahl, 2004; Mumme et al., 2006a,b; Schutter and Dick, 2002; van Gestel et al., 1996). Accordingly, we found $^{13}$C-PLFA derived from root- and residue-C to be different between the two soil microenvironments analyzed in this study. Yet, we did not find consistently greater microbial processing to fungi. Furthermore, the standard tillage regime applied in the cropping systems (~14 passes to 30 cm depth) may also have diminished fungal PLFA biomass and led to the dominance of bacterial PLFA in the soil microbial community (Frey et al., 1999; Six et al., 2006). Lastly, with the addition of ‘high’ quality (low C:N ratio) hairy vetch roots and residues, a change in fungal:bacterial $^{13}$C-PLFA ratios might not have occurred during our relatively short observation period of the decomposition dynamics (134 days after incorporation); however, longer term observations might have revealed changes in the ratios.
of root- and residue-derived PLFA-C in the microaggregate fraction compared to the silt-and-clay. Rather, $^{13}$C-PLFA concentrations in the silt-and-clay were higher than in the microaggregates for a number of bacterial biomarkers in the low-input and organic systems (Figs. 3 and 4), and only fungal $^{13}$C-PLFA concentrations were greater in the microaggregate than the silt-and-clay in all systems. Finding greater $^{13}$C-PLFA concentrations in the silt-and-clay particles is probably due to the slightly higher root- and residue-derived C concentrations associated with this fraction compared to the microaggregate fraction (Kong and Six, 2010). Although the cropping systems did not have an overall effect on the microbial communities, the interaction between soil microenvironments and crop management appeared to have a greater impact on C processing within the microbial community. With more $^{13}$C-enriched PLFAs a15:0, 18:1u7, and 16:0 in the silt-and-clay than the microaggregates of the low-input and organic systems, there were greater differences in the composition of microbial communities between the two soil microenvironments in the low-input and organic than in the conventional system. Carbon processing by microbial groups within the two soil microenvironments may have been greater in the low-input and organic systems because these systems have regular cover crop inputs for the last 13 years and more than in the conventional system, where only mineral fertilizer-N was applied to the conventional system. Although the low values of the fungal:bacterial PLFA biomass ratios in both microaggregates and silt-and-clay implied that bacteria were prevalent in both microenvironments, our data suggested that the microaggregates also provided favorable conditions for fungal activity. The greater fungal:bacterial PLFA biomass ratio of the microaggregates (0.23) than the silt-and-clay (0.16) suggested that microaggregates contained substrates that were more suitable for fungal than bacterial decomposition. Microaggregates may have been less restrictive for the growth of fungal hyphae than the silt-and-clay particles, as fungal hyphae has been shown to enmesh microaggregates into larger aggregates (Tisdall and Oades, 1982). Moreover, we did not find greater processing of hairy vetch root- and residue-C inputs by actinomycete, Gram-positive, and Gram-negative bacteria in the silt-and-clay microenvironment. In addition, total fungal PLFA-C derived from both root- and residue-C was generally greater in the microaggregate than the silt-and-clay. Simpson et al. (2004) attributed the greater stabilization of microbial-derived C in no-tillage than conventional tillage treatments to preferential accumulation of fungal-C within microaggregates contained within microaggregates. Using ratios of glucosamine and muramic acid, Guggenberger et al. (1999) showed that, in no-tillage soils, fungi are dominant in >53 µm-sized aggregates, but not in smaller particles.

4.4. Conclusions

This study made progress towards describing the relationship between long-term C sequestration at the cropping system-scale and C cycling within microbial groups in soil aggregates. We found nearly four times more root-C than residue-C assimilated into PLFA-C, which supports results from our previous study that showed greater relative root-C contributions to overall SOM stabilization than residue-C contributions. When corrected for $^{13}$C content, however, our data show that microbial communities processing root- and residue-C within the three cropping systems were not different, even though total PLFA biomass was higher in the organic than in the conventional and low-input systems. Therefore, our first hypothesis (i.e., that microbial communities process root-C more rapidly than residue-C, thereby, leading to more C sequestration in organic than conventional and low-input cropping systems) was not corroborated and differences in microbial community composition and function did not provide a mechanistic explanation for the trends in long-term C sequestration among the cropping systems. Our second hypothesis was also not corroborated because microbial processing of root- and residue-C was not consistently greater in the microaggregate than in the silt-and-clay microenvironment. However, we found higher fungal:bacterial PLFA biomass ratios, along with more fungal PLFA-C derived from both root- and residue-C in the microaggregate than the silt-and-clay. This suggested that more residue- and root-C was incorporated into fungal PLFAs within the microaggregates, making the microaggregate a relatively unique microenvironment for fungal activity.

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