2001-2006 Mission
Kearney Foundation of Soil Science: Soil Carbon and California's Terrestrial Ecosystems
Final Report: 2004207, 1/1/2005-12/31/2006

Linking Cover Crop C Sequestration to Microbial Community Function within Soil Microenvironments

Johan Six[1*], Krassimira Hristova[2], and Angela Kong[1]

Summary

The global objective of this project is to elucidate how the interrelationships between cover crop-C input, soil microenvironments, and microbial community structure and function control C sequestration in cover crop systems across a gradient of nutrient treatments. To address this objective, we conducted a field study in three long-term (13 years) maize (*Zea mays* L.) –tomato (*Lycopersicon esculetum*) cropping systems that differed in nutrient input treatments: *conventional* (synthetic fertilizer only), *low-input* (alternating synthetic fertilizer and cover crop) and *organic* (cover crop and organic amendments). In each cropping system, *Vicia dasycarpa* (hairy vetch) was grown and $^{13}CO_2$-labeled at regular intervals during the 2006 cover crop growing season. At the time of incorporation, ^{13}C-labeled aboveground biomass was manually incorporated into unlabeled belowground biomass, and vice versa. Soil samples collected across the subsequent maize-growing season were analyzed to study, first, the decomposition dynamics of the aboveground versus belowground biomass, and second, to link the decomposition processes to microbial activity within specific soil microenvironments.

Results from the decomposition study showed more rapid decomposition of belowground compared to aboveground cover crop biomass across the three cropping systems. Decomposition of belowground biomass did not differ at the beginning of the growing season; however, by the 3rd sampling period, incorporation of belowground biomass was greatest in the organic system (850.0 kg C_{new} ha^{-1}), with the low-input rotation (494.1 kg C_{new} ha^{-1}) showing nominally intermediate, but similar decomposition dynamics to the conventional system, where incorporation was the lowest (322.4 kg C_{new} ha^{-1}). Trends in aboveground residue decomposition were nearly the opposite to those of the belowground biomass decomposition in the organic system was lowest in the beginning and in the end of the season (117.5 kg C_{new} ha^{-1}), while incorporation of the aboveground residues into the conventional system was greatest at the 4th (final) sampling period (348.9 kg C_{new} ha^{-1}).

We successfully traced the assimilation of ^{13}C-labeled residue and belowground biomass into the phospholipid fatty acids (PLFAs) extracted from the microaggregate and silt-and-clay soil organic matter fractions, which were isolated from soils incorporated with either ^{13}C-labeled aboveground or belowground cover crop (soils from the decomposition study). The microbial community active in decomposing the aboveground residues was different from the community utilizing the belowground cover crop biomass. ^{13}C-PLFA profiles also revealed differences between the community structure associated with microaggregates, compared to the silt-and-clay

[1]Department of Plant Sciences, University of California, Davis

[2]Land, Air, and Water Resources Department, University of California, Davis

fraction. Results from this study support the findings from the decomposition study and also suggest that microaggregate and silt-and-clay structures are unique microenvironments, with disparate cover crop-C dynamics.

Objectives

The overall objective of this project was to elucidate how the interrelationships between cover crop-C input, soil microenvironments, and microbial community structure and function control C sequestration in cover crop systems. Hence, we investigated how the microbial community structure mediates the turnover and subsequent C stabilization of belowground and aboveground cover crop-C across various soil microenvironments. To achieve our global objective, the following specific objectives were formulated:

Objective$_1$: Compare the aboveground versus belowground influence of cover crops on the formation of soil microenvironments and the development of the microbial community structure.

Objective$_2$: Link microbial community structure and activity within soil microenvironments to the turnover and stabilization of cover crop-derived C.

Objective$_3$: Evaluate the impact of long-term agroecosystem management on short-term cover crop C cycling through the microbial community and soil microenvironments.

Approach and Procedures

Field Study

Since 1993, the Center for Integrated Farming Systems, formerly known as the Long-Term Research on Agricultural Systems, has conducted research on the sustainability of conventional and alternative cropping management practices. We conducted our field study in three cropping systems (*table 1*), which vary in nitrogen levels and source: i) *conventional* (synthetic fertilizer only; conventional), ii) *low-input* (synthetic fertilizer and cover crop; low-input) and iii) *organic* (cover crop and composted manure; organic). Experimental plots (4.6m x 9.1m) were established in these cropping systems, which are represented by three 0.4-ha field replicates. At the start of the 2006 cover crop growing season (October 2005), Time-Zero whole soil samples (4-cm dia.; 0-15 cm) were taken from each of the replicate plots of the conventional, low-input, and organic systems to measure baseline total soil C concentrations. Two 1-m^2 sub-plots were established within each of the experimental plots. Subsequently, a winter legume cover crop (*Vicia dasycarpa*) was sown into each experimental plot. To address objective$_1$, only one of the two 1-m^2 sub-plots was pulse-labeled with $^{13}CO_2$ (99 atom%) over the cover crop growing season. At the time of cover crop incorporation, the aboveground biomass of the $^{13}CO_2$ labeled 1-m^2 sub-plot was incorporated into the unlabeled sub-plot, and the aboveground biomass of the unlabeled sub-plot was incorporated into the ^{13}C-labeled sub-plot. Four soil samples were taken from each of the sub-plots throughout the maize growing season that followed the cover crop

growing season. Due to unanticipated obstacles in the 2006 cover crop growing season, four 0.13-m^2 sub-plots were established within the experimental plots in the 2007 season and were individually $^{13}CO_2$-labeled (99 atom%) across the cover crop season. Soil cores + cover crop above- and belowground plant samples (0.04 m^3) were taken 24 hours after these $^{13}CO_2$ labeling sessions.

Table 1. *Characteristics of long-term maize-tomato cropping systems at the Center for Integrated Farming Systems.*

Maize-Tomato Cropping System	Even Years of Cropping	Odd Years of Cropping
Conventional	fertilized irrigated maize	fertilized irrigated tomato
Low-input	winter cover crop then irrigated maize	fertilized irrigated tomato
Organic	winter cover crop then irrigated maize with compost and no pesticides	winter cover crop then irrigated tomato with compost and no pesticides

Laboratory Analyses

Whole soil samples collected at Time-Zero and the 1st, 2nd, 3rd, and 4th/final sampling periods were separated into three soil organic matter (SOM) fractions by wet sieving through a microaggregate isolator, according to the methodology outlined in Six et al. (2000): i) macroaggregates (>250 μm), ii) microaggregates (53-250 μm), and iii) silt-and-clay (<53 μm). Subsamples of the SOM fractions and of the whole soil samples were ground and analyzed for elemental and isotopic C concentrations using a PDZ Europa ANCA CN analyzer coupled to a PDZ Europa 20-20 IRMS (Cheshire, UK).

Phospholipid fatty acids were extracted from the microaggregate and silt-and-clay fractions using our modifications of the method of Bossio and Scow (1995). Identification, quantification, and $\delta^{13}C$ signature measurement of the PLFAs were determined using a Thermo gas chromatograph-combustion-isotope ratio mass spectrometer (GC-C-IRMS) system composed 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 a J&W DB-5 column.

Results

Baseline Whole Soil Organic C (SOC) Measurements

At Time-Zero, whole soil C concentrations were higher in the organic cropping system (20.2 Mg SOC ha^{-1}) than in both the conventional (15.6 Mg SOC ha^{-1}) and the low-input (15.3 Mg SOC ha^{-1}) cropping systems (p<0.05; data not shown). After 13 years of continuous crop management, SOC sequestration in the organic system (5.70 Mg SOC ha^{-1}) was greater than the amount of SOC sequestered by the conventional and low-input systems (570 and -340 kg SOC

ha^{-1}, respectively), at the $p<0.05$ significance level (*fig. 1*). The nominal gain and loss of SOC since 1993 in the conventional and low-input cropping systems, respectively, were not different.

Across the maize growing season, SOC levels in the three cropping systems were generally greatest in the organic system (20.6 Mg C ha^{-1}, averaged across the season) and similar in the low-input and conventional systems (15.0 Mg C ha^{-1} and 16.3 Mg C ha^{-1}, respectively averaged across the season) ($p<0.05$; *fig. 2*). Aside from a slight decrease in SOC content of all cropping systems at the 1st sampling period, the SOC levels did not change within the cropping systems over the course of the season.

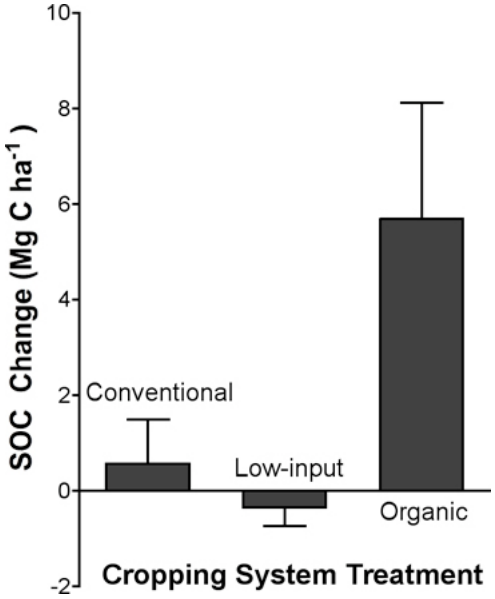


Figure 1. Soil organic C sequestered in the conventional, low-input, and organic maize-tomato cropping systems after 13 years of continuous cropping at the Center for Integrated Farming Systems. Error bars indicate the standard errors of the means.

Decomposition Study of ^{13}C-labeled Cover Crop Biomass

Overall, belowground cover crop biomass decomposed faster and more belowground biomass was incorporated into the three cropping systems compared to the aboveground cover crop residue (*fig. 3*). More specifically, ^{13}C-labeled belowground biomass measured in the whole soil, e.g., 494.1 kg C_{new} ha^{-1} in the low-input rotation at the 3rd sampling period, nearly doubled the concentrations of ^{13}C-labeled aboveground biomass measured in the three cropping systems, e.g., 235.7 kg C_{new} ha^{-1} in the low-input rotation at the 3rd sampling period (*fig. 3*).

Decomposition of belowground biomass did not differ between cropping systems at the beginning of the growing season; however, by the 3rd sampling period, incorporation of belowground biomass was greatest into the organic system (850.0 kg C_{new} ha^{-1}), with the low-input rotation (494.1 kg C_{new} ha^{-1}) showing nominally intermediate, but similar decomposition dynamics to the conventional system, where incorporation was the lowest (322.4 kg C_{new} ha^{-1}).

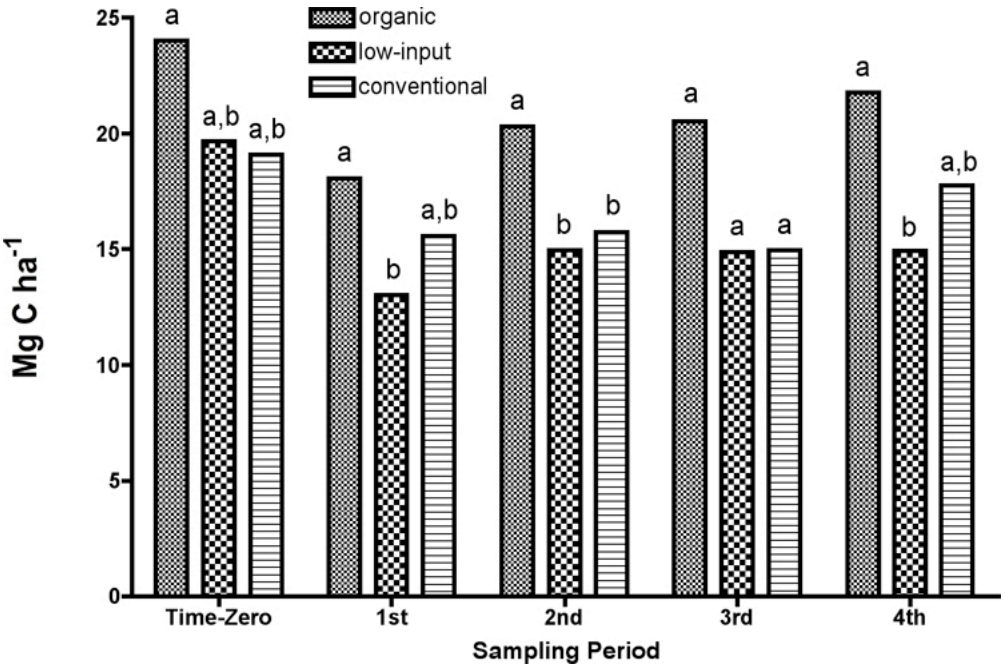


Figure 2. Whole soil carbon content in the organic, low-input, and conventional cropping systems, at Time-Zero and across the four sampling points in the 2006 maize growing season. Letters above the bars indicate significant differences (p<0.05) between the cropping systems, within a sampling period.

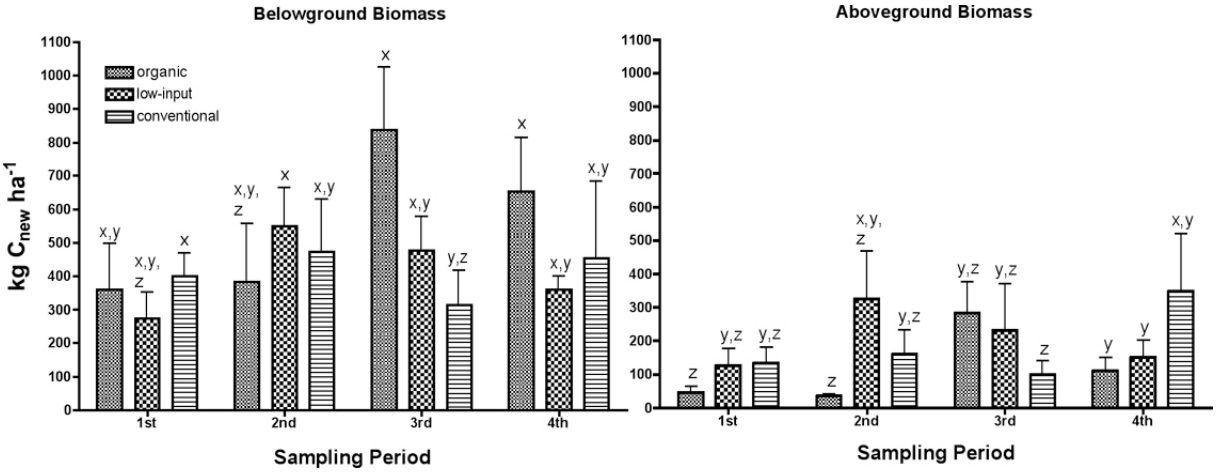


Figure 3. Decomposition dynamics of aboveground *Vicia dasycarpa* (AG) versus belowground (BG) *V. dasycarpa* as shown with ^{13}C (kg C_{new} ha^{-1}) measured in whole soil of the organic, low-input, and conventional cropping systems. Letters above the bars indicate significant differences between the above- and belowground biomass, within a sampling period (p<0.05).

Trends in aboveground residue decomposition were nearly the opposite to those of the belowground biomass in that decomposition in the organic system was lowest in the beginning and in the end of the season (117.5 kg C_{new} ha^{-1}), while incorporation of the aboveground

residues into the conventional system was greatest at the 4th (final) sampling period (348.9 kg C_{new} ha^{-1}).

^{13}C-PLFA in Microenvironments

Based primarily on the PLFAs extracted from the microaggregate and silt-and-clay fractions of the 1st sampling period, microbial biomass in the cropping systems generally decreased according to the following: organic > conventional > low-input (*fig. 4*). Due to high variability, no differences were found in total biomass between the cropping system soils enriched with ^{13}C-labeled aboveground cover crop residue. In contrast, total biomass of samples enriched with ^{13}C-labeled belowground biomass was highest in the organic system (46.1 nmoles g^{-1} dry soil), intermediate in the conventional system (41.6 nmoles g^{-1} dry soil), and lowest in the low-input system (33.7 nmoles g^{-1} dry soil).

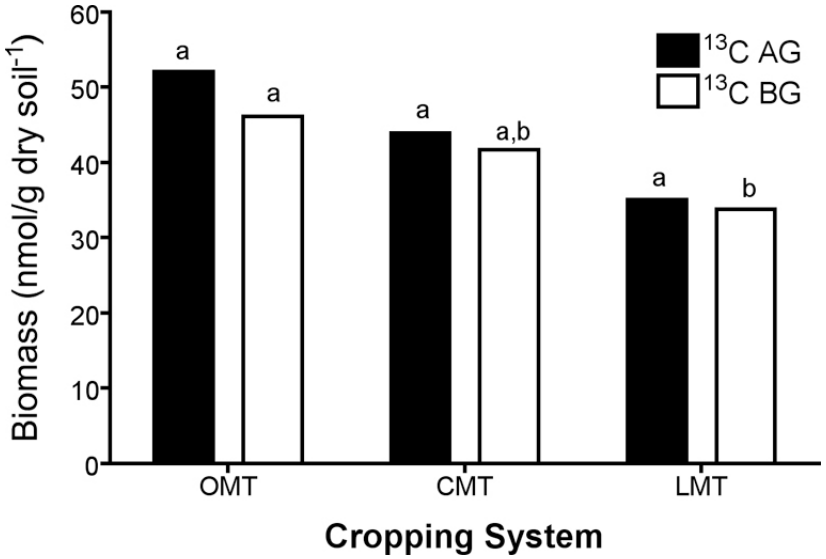


Figure 4. Total microbial biomass (nmoles g^{-1} dry soil) of microaggregate and silt-and-clay fractions isolated from soils either ^{13}C-enriched with aboveground or belowground *Vicia dasycarpa*, in organic, low-input, and conventional cropping systems. Letters above the bars indicate significant differences between each treatment, within the biomass type (AG=Aboveground; BG=Belowground; (p<0.05)).

Correspondence analysis (CA) (CANOCO version 4.0, Microcomputer Power, Inc. Ithaca, NY) of PLFA-C in microenvironment samples explained a total of 61.1% of the variation between two axes and separated the samples based on soil sampling period (*fig. 5*). Meanwhile, a second CA of ^{13}C-PLFA in the samples indicated that PLFA profiles of samples ^{13}C-labeled with aboveground cover crop biomass are different from PLFA profiles from ^{13}C-labeled belowground biomass samples (*fig. 6*). Axes 1 and 2 of the latter CA explained 29.4 and 15.2% of the variation, respectively, and separated the samples by both SOM fraction (microaggregate versus silt-and-clay) and the type of cover crop biomass incorporated (*fig. 7*). One reason for the overlap in the ^{13}C-belowground biomass-silt-and-clay samples with its microaggregate

counterpart is that the dataset is not yet complete, and a third axis may create more distinct separations among the fractions.

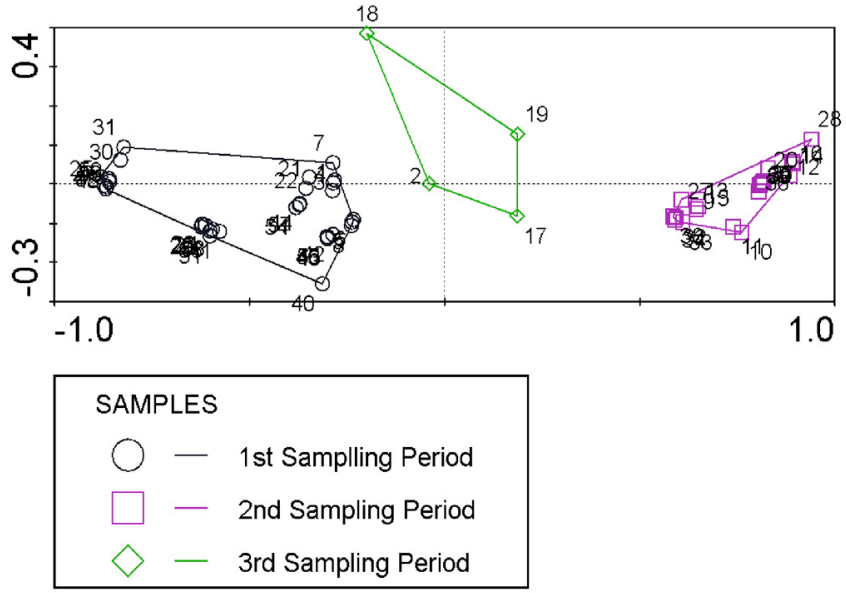


Figure 5. Correspondence analysis (CA) ordination plot of PLFA-C profiles for microaggregate and silt-and-clay fractions isolated from soils collected from organic, low-input, and conventional cropping systems, at three sampling periods after *Vicia dasycarpa* incorporation. Axes are scaled to represent proportional significance of Axis 1 and 2, which explain 41.8 and 19.3% of the total variation, respectively. Polygons indicate major groupings of samples from either aboveground (13C AG) or belowground (13C BG) ^{13}C-labeled *V. dasycarpa*.

Discussion

Long-term Crop Management Effects on SOC Sequestration

Increased N fertilization has been correlated to increased SOC sequestration (Campbell et al. 1991; Dumanski et al. 1998), which can lead to better plant growth and increased crop productivity. Despite receiving a relatively high N fertilization rate, the conventional system did not show the highest SOC stocks of the three cropping systems after 13 years of cropping. This supports findings from recent studies showing that, while synthetic fertilizer-N may increase crop residue returns, N fertilization has a net negative effect on SOC sequestration (Omay et al. 1997; Halvorson et al. 2002; Russell et al. 2005). The conventional and organic systems both received high rates of N additions (280 and 473 kg N ha^{-1} yr^{-1}, respectively), yet the organic system, where solely organic amendments were applied, sequestered disproportionately more SOC than the amount of C input it received compared to the other systems. The greater long-term protection and stabilization of C derived from the cover crop within aggregate structures may have fostered the gradual accumulation of a large pool of soil C in the organic system, compared to the conventional and low-input cropping systems.

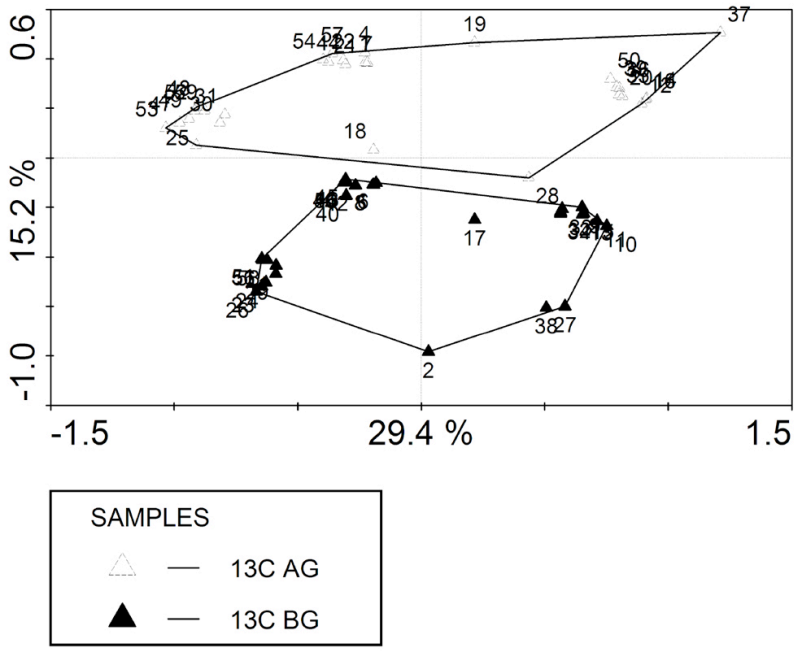


Figure 6. CA ordination plot of ^{13}C-enriched fatty acid methyl esters derived from ^{13}C-labeled *Vicia dasycarpa* incorporated into mineral soil fractions isolated from soils collected from organic, low-input, and conventional cropping systems. Axis 1 and 2 explain 29.4 and 15.2% of the total variation, respectively. Axes are scaled to represent proportional significance of Axis 1 and 2. Samples are grouped into either aboveground (13C AG) or belowground (13C BG) ^{13}C-labeled *V. dasycarpa.*

Furthermore, the addition of solely organic amendments to an organic system may induce greater protection and stabilization of C than low-input and conventional systems in the long-term. In this study, the low-input system may not have received the long-term SOC benefits of its organic amendments, due to its biannual additions of a synthetic fertilizer, which may have increased the decomposition of SOM-C.

Cover Crop Biomass Decomposition

The intermediate levels of ^{13}C-labeled cover crop biomass (aboveground and belowground) measured in the low-input cropping system were expected, since this system received an intermediate level of nitrogen and a cover crop in alternate years (following the tomato rotation). This system likely houses a microbial community that is adapted to the processing and cycling of cover crop biomass, but is not as robust as the community in the organic system, in which a cover crop is grown annually. Because the 2006 incorporation of a cover crop into the conventional cropping system was the first in its 13 years of continuous management, the unchanging amount of ^{13}C-labeled biomass incorporated into this system was also not surprising. The greater contribution to SOM from the belowground biomass versus the aboveground residue is probably due to the greater contact between root-derived material and the soil matrix, which

can lead to a stronger interaction and faster stabilization of root-derived C in soil. In addition, during cover crop growth, active crop roots are continuously releasing a range of organic compounds (i.e., carbohydrates, carboxylic acids, and amino acids) into the rhizosphere (Oades 1978) in the form of root exudates and sloughed-off root material, which are likely more readily available C sources for microorganisms than incorporated residues.

Microbial Community Structure and Activity in Microenvironments

Several studies have shown that soil aggregates represent an ecological niche whose chemical and physical properties may contribute to the heterogeneous distribution of microorganisms and their activity among aggregates of different sizes. Different population numbers and structure of denitrifiers (Seech and Beauchamp 1988, Philippot et al. 1997), rhizobia (Mendes and Bottomley 1998), and diazotrophic communities (Poly et al. 2001) among aggregate size classes have been reported. Preliminary ^{13}C-PLFA results from this study have started to link the processing of cover crop biomass to different microbial communities, corresponding to either aboveground or belowground biomass (*fig. 6*). Furthermore, the CA biplot of ^{13}C-PLFA further support the notion that different soil microenvironments (e.g., microaggregates versus silt-and-clay fraction) support different microbial communities, which process C dissimilarly (*fig. 7*).

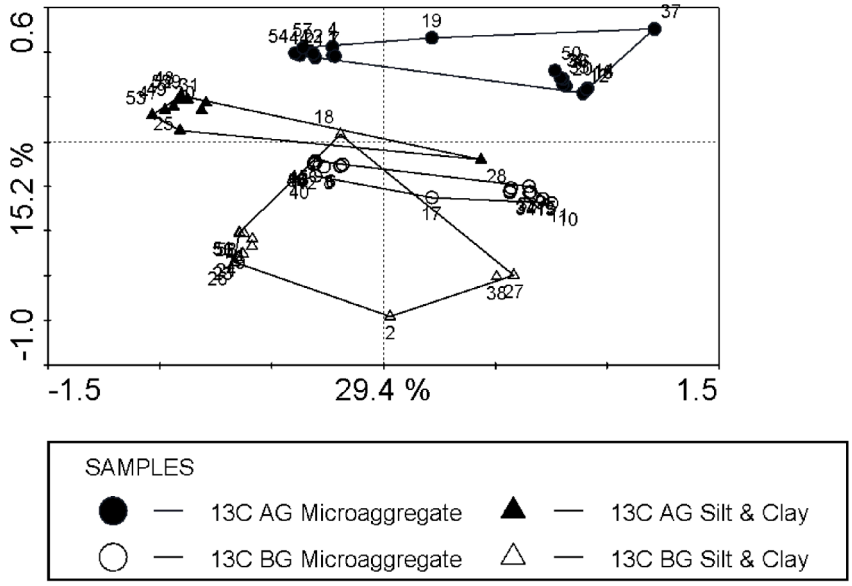


Figure 7. CA ordination plot of ^{13}C-enriched fatty acid methyl esters derived from ^{13}C-labeled *Vicia dasycarpa* incorporated into mineral soil fractions isolated from soils collected from organic, low-input, and conventional cropping systems. Axis 1 and 2 explain 29.4 and 15.2% of the total variation, respectively. Axes are scaled to represent proportional significance of Axis 1 and 2. Polygons indicate major groupings of samples into aboveground (*13C AG*) or belowground (*13C BG*) ^{13}C-labeled *V. dasycarpa*, by soil fraction (*Microaggregate* or *Silt & Clay*).

References

Bossio, D.A., and K.M. Scow. 1995. Impacts of carbon and flooding on soil microbial communities: phospholipids fatty acid profiles and substrate utilization patterns. *Applied and Environmental Microbiogy* 61:4043-4050.

Campbell, C.A., K.E. Bowren, M. Schnitzer, R.P. Zentner, and L. Townley-Smity. 1991. Effect of crop rotations and fertilization on soil biochemical properties in a thick black chernozem. *Canadian Journal of Soil Science* 71:377-387.

Dumanski, J., R.L. Desjardins, C. Tarnocai, C. Monreal, E.G. Gregorich, V. Kirkwood, and C.A. Campbell. 1998. Possibilities for future carbon sequestration in Canadian agriculture in relation to land use changes. *Climatic Change* 40:81-103.

Halvorson, A.D., B.J. Wienhold, and A.L. Black. 2002. Tillage, nitrogen, and cropping system effects on soil carbon sequestration. *Soil Science Society of America Journal* 66:906-912.

Mendes, I.C, A.K. Bandick, R.P. Dick, and P.J. Bottomley. 1999. Microbial biomass and activities in soil aggregates affected by winter cover crops. *Soil Science Society of America Journal* 63:873-881.

Oades, J.M. 1978. Mucilages at the root surface. *Journal of Soil Science* 29:1-16.

Omay, A.B., X.W. Rice, L.D. Maddux, and W.B. Gordon. 1997. Changes in soil microbial and chemical properties under long-term crop rotation and fertilization. *Soil Science Society of America Journal* 61:1672-1678.

Phillipot, L., P. Renault, J. Sierra, C. Henault, A. Clays-Josserand C. Chenu, R. Chaussod, and R. Lensi. 1997. Dissimilatory nitrite-reductase provides a competitive advantage to Pseudomonas spp. RTC01 to colonise the centre of soil aggregates. FEMS Microbiology Ecology 21:175-185.

Poly, F., L. Ranjard, S. Nazaret, F. Gourbiere, and L.J. Monrozier. 2001. Comparison of nifH gene pools in soils and soil microenvironments with contrasting properties. Applied Environmental Microbiology 67:2255-2262.

Russell, A.E., D.A. Laird, T.B. Parkin, and A.P. Mallarino. 2005. Impact of nitrogen fertilization and cropping system on carbon sequestration in Midwestern Mollisols. *Soil Science Society of America Journal* 69:413-422.

Seech, A.G., and E. Beauchamp. 1988. Denitrification in soil aggregates of different sizes. *Soil Science Society of America Journal* 52:1616-1621.

Six J., E.T. Elliott, and K. Paustian. 2000. Soil macroaggregate turnover and microaggregate formation: A mechanism for C sequestration under no-tillage agriculture. *Soil Biology and Biochemistry* 32: 2099-2103.

This research was funded by the Kearney Foundation of Soil Science: Soil Carbon and California's Terrestrial Ecosystems, 2001-2006 Mission (http://kearney.ucdavis.edu). The Kearney Foundation is an endowed research program created to encourage and support research in the fields of soil, plant nutrition, and water science within the Division of Agriculture and Natural Resources of the University of California.