

## Plant Species Composition, Soil Biology, and Carbon Storage in Grasslands

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### Summary

The primary focus of this project was on plants with different rooting attributes, effects of roots on microbial communities, and contribution of root-related processes to soil C retention, but fates of aboveground litter were also examined. In a project comparing deep soil profiles, there were very few differences between annual grassland and restored perennial grassland in their total soil C, soil microbial C, CO<sub>2</sub> efflux from soil, soil CO<sub>2</sub> concentrations in the soil atmosphere, and soil microbial community composition based on phospholipid fatty acid (PLFA) profiles, although root length of the native perennials were slightly lower at the surface and greater at lower depths than in the annual grass-dominated areas. The two years of tillage to reduce non-native annuals prior to planting perennials thus did not have a long-lasting effect on soil C pools, compared to the annual grassland starting point. Long-term tillage for six years with no plants present, however, resulted in much lower C pools, lower decomposition of litter in litterbags, and changes in microbial community composition, e.g., absence of a PLFA fungal biomarker. Root effects also tended to be minor in another project that compared “bare” treatments (plants were removed) vs. “plant” treatments (the perennial bunchgrass, *Nassella pulchra*, was present), although some sites such as relict perennial grassland showed higher C pools and different PLFA in the “plant” treatment. In contrast, other related projects showed that aboveground litter deposition had pronounced effects on the soil surface layer, including microbial biomass, activity, and community composition. Addition of C<sub>4</sub> litter resulted in rapid assimilation of litter-derived C into the microbial biomass. Litterbags indicated that plant species composition and litter placement have a potentially large effect on C flows. To further address the effects of litter and plant species composition, a field microcosm experiment was initiated in December 2002 to compare the effects of site litter of annual plants vs. perennial grass litter, annual lupin plantings, and added phosphorus. Further study is also needed to understand the unexpectedly large difference in soil responses to root vs. shoot inputs.

### Objectives

Our goal was to better understand how plant species composition affects soil C dynamics and C storage in grasslands. The primary focus was on plants with different rooting attributes, effects of roots on microbial communities, and contribution of root-related processes to soil C retention in grasslands of different management histories. Long-lived perennial grasses can construct much deeper roots than annual grasses, whose life cycle is limited to a few months during the cool, moist period of the year (Amundson and Smith 1988; Holmes and Rice 1996). The context was a comparison of grassland soils: restored native perennial bunchgrass grassland, annual grassland,

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and recently-tilled, fallow soil and, additionally in one project, relict perennial grassland and an agricultural site for producing seed of native perennial grasses. Our objectives were to:

1. Compare soil C pools, microbial activity, plant root distribution, and above- and belowground litter quality in grassland soils with different management histories, and different assemblages of plant species;
2. Determine the fate of root-derived material from different plant species in microcosm studies using intact cores of grassland soils;
3. Characterize some aspects of microbial communities in the rhizosphere of different plant species, e.g., PLFA profiles, and relate to C and N dynamics in the microcosm studies;
4. Provide a possible management scenario that enhances C storage in California grasslands based on the experimental outcomes.

These objectives are slightly modified due to 1) our findings that soil C dynamics were only slightly affected by rooting patterns with depth in restored perennial grassland (Jackson et al. 2003), 2) interest in C fates of surface litter of different plant species, and 3) availability of new isotopic methods, i.e., determination of  $^{13}\text{C}$  in microbial biomass (Potthoff et al. 2003) that caused us to change the experimental design from labeling with enriched  $^{13}\text{C}$  to tracking changes in natural abundance of  $^{13}\text{C}$  after adding aboveground residues of  $\text{C}_4$  grasses with less negative  $\delta^{13}\text{C}$  than the local vegetation and soil.

## Approach and Procedures

*Comparison of soil C, microbes, and plant roots in grassland soils with different management histories.* The grassland sites were located on level areas that were farmed between 1865 and 1937 at the UC Hastings Natural History Reservation in the foothills of the Santa Lucia Mountains in Upper Carmel Valley. From 1937 to 1996, the site was annual grassland. We established, in collaboration with Dr. M. Stromberg, different management treatments for grasslands beginning in 1996: 1) tilled soil that has been continuously plant-free using tillage and Round-up™ herbicide; 2) annual grassland; and 3) restored perennial grassland that was planted in Fall 1997, after tilling annual grassland and planting with the native bunchgrasses, *Nassella pulchra* and *Elymus glaucus*. All treatments are in close proximity on Gorgonio sandy loam derived from granitic and alluvial parent material.

In the fourth year after planting native perennial grasses, total soil C and nitrogen (N), microbial biomass C by chloroform fumigation extraction (Vance et al. 1987), microbial respiration,  $\text{CO}_2$  concentrations in the soil atmosphere, surface efflux of  $\text{CO}_2$  in the headspace of capped cylinders, and root distribution (0-15, 15-30, 30-60 and 60-80 cm depths) were measured at the peak of spring growth in April 2002 (Potthoff et al. 2005). Gas samples were collected in vacutainers (Becton Dickinson, Franklin Lakes, NJ) and analyzed using a  $\text{CO}_2$ -analyzer (Horiba PIR-200, Riverside, CA) with IR-detection. A comparison was made between restored perennial grassland, untreated annual grassland and tilled plots maintained without plants by rototillage and herbicide.

In Spring 2003, sampling of  $\text{CO}_2$  concentrations in the soil atmosphere and temperature at these same depths was conducted five times from March until June. Thin steel tubes, with perforations along 4 cm of their length at the center of the designated depth, were pushed into soil. Tubes were sealed with rubber septa to sample soil gas at the different depths. The

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perforation of the steel tubes was protected from soil contamination with fine-mesh steel screen. The upper soil layer was sampled using canulated needles (7.5 cm depth). CO<sub>2</sub> surface efflux was determined using the closed chamber method modified from Rolston (1986). Analyses were carried out as described for the soil gas CO<sub>2</sub> concentrations above. Soil was cored for gravimetric moisture content at all depths on all dates in areas at least 1 m away to avoid disturbing the sampling tubes.

*Fungal markers in grassland soils with different management histories.* To determine the role of fungi in soil C transformations, two methods were utilized to compare the grassland soils, using soil obtained from the sampling described above: ergosterol, a fungal byproduct, and fungal phospholipids fatty acids (PLFA) markers. Methods were: PFLA (Bossio and Scow 1995); ergosterol (Djakirana et al. 1996); and Canonical Correspondence Analysis (CCA) to analyze the data.

*Root effects on soil C dynamics and microbial communities.* The relationship between C availability, N availability, microbial community composition with PLFA, and root-derived C was tested by comparing soil under native perennial bunchgrasses (*Nassella pulchra*) and adjacent bare soil areas that were cleared in early fall in a relict perennial grassland, a restored perennial grassland, and a perennial grass agriculture site on soils from granitic and schistose alluvium in Upper Carmel Valley (Steenwerth 2003). An exotic annual grassland site was also included, since perennial bunchgrasses exist within a matrix of annual grassland. The comparison of soil under plants (plant) and bare soils (bare) occurred on Oct. 20, 2000, and Feb. 19 and March 19, and the analyses were completed in 2003. Soil samples were taken at 0-12 cm, where root length density is highest in the profile. Lab methods are described above, and in addition, N availability was measured with KCl extracts and anaerobic incubation for potentially mineralizable N. In the late spring, when annual plants were approaching senescence, plant community diversity and relative cover were assessed by the point frame method in the grassland plots. PLFA methods and analysis are described briefly above.

*Carbon flow using C<sub>4</sub> grass residue with and without legumes.* To investigate the turnover and soil incorporation of surface inputs of perennial grass litter, in December 2002, PVC microcosms (30 cm deep x 30 cm diameter cylinders) were pushed 25 cm deep into the soil at one of restored perennial grasslands. Every cylinder encircled a 6-year-old *Nassella pulchra* plant. Most of the existing litter in the cylinders was that of annual grasses. In half of the cylinders, existing C<sub>3</sub> litter (≈30 g per cylinder) was removed, and 50 g litter of *Bouteloua gracilis*, corresponding to 700 g m<sup>-2</sup> was replaced. *Bouteloua gracilis* is a C<sub>4</sub> plant that had higher natural abundance of <sup>13</sup>C in its tissue (δ<sup>13</sup>C = -13.96) compared to *Nassella pulchra*, a C<sub>3</sub> plant (δ<sup>13</sup>C = -27.85). They have similar C/N ratio: 114 for *Nassella pulchra* and 99 for *Bouteloua gracilis*. Germinated seeds (≈100 per cylinder) of the native annual legume, *Lupinus bicolor* (miniature lupine), were scattered on the soil surface of half of the cylinders. Phosphorus was added to half of the cylinders (31 kg P ha<sup>-1</sup> as Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>·H<sub>2</sub>O in March 2003) to ensure that P was not limiting, especially for legumes, yet even without the fertilizer addition, soil concentrations were already reasonably high (25 μg P g<sup>-1</sup> soil at 0-15 cm with the Bray method). The eight treatments were each replicated five times in a random design:

- 1) C<sub>4</sub> (*B. gracilis*) litter, -*Lupinus*, -P
- 2) C<sub>4</sub> (*B. gracilis*) litter, +*Lupinus*, -P
- 3) C<sub>4</sub> (*B. gracilis*) litter, -*Lupinus*, +P

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- 4) C<sub>4</sub> (*B. gracilis*) litter, +*Lupinus*, +P
- 5) C<sub>3</sub> (Annual plants + *N. pulchra*) litter, -*Lupinus*, -P
- 6) C<sub>3</sub> (Annual plants + *N. pulchra*) litter, +*Lupinus*, -P
- 7) C<sub>3</sub> (Annual plants + *N. pulchra*) litter, -*Lupinus*, +P
- 8) C<sub>3</sub> (Annual plants + *N. pulchra*) litter, +*Lupinus*, +P

Soil sampling occurred on Dec. 8, 2002, and March 28, and April 16, 2003, at 0-7.5 cm, 7.5-15 cm, and 15-30 cm. Plant biomass was assessed at the senescence of vegetation on May 30, 2003, by visual estimates of aboveground biomass that were related to a set of clipped vegetation samples of known biomass. The same approach was used to estimate lupine biomass.

Organic C and  $\delta^{13}\text{C}$  in the K<sub>2</sub>SO<sub>4</sub> extracts was measured using diluted extracts with CO<sub>2</sub> passed from an "OI Analytical" TOC analyzer in line with a PDZ Europa 20-20 isotope ratio mass spectrometer at the UC Davis Stable Isotope Facility. Total organic C was determined according to the method of Wu et al. (1990). Soil microbial biomass C was calculated from the relationship: biomass C =  $E_C/k_{EC}$  ( $E_C$  = [organic C extracted from fumigated soil] - [organic C extracted from non-fumigated soil];  $k_{EC}$ =0.45) (Wu et al. 1990; Joergensen 1996). From the outlet of the TOC analyzer gas samples were directed to the mass spectrometer for  $^{13}\text{C}$  access (Potthoff et al. 2003; St. Jean 2003).

Litter-derived C was quantified for soil organic C and soil microbial biomass C in the cylinders with *Bouteloua gracilis* litter. The isotopic composition of a sample was calculated as follows:

$$\delta^{13}\text{C}_{\text{SA}} (\%) = ({}^{13}\text{C}_{\text{SA}} / {}^{12}\text{C}_{\text{SA}} - {}^{13}\text{C}_{\text{PDB}} / {}^{12}\text{C}_{\text{PDB}}) / ({}^{13}\text{C}_{\text{PDB}} / {}^{12}\text{C}_{\text{PDB}}) \times 1000$$

where C<sub>SA</sub> is the C of the sample and C<sub>PDB</sub> is the C of the PDB standard. The litter-derived portion (%) of C in soil organic carbon (SOC) was calculated as follows:

$$C_{\text{LT}} = (\delta^{13}\text{C}_{\text{S}} - \delta^{13}\text{C}_{\text{SL}}) / (\delta^{13}\text{C}_{\text{BG}} - \delta^{13}\text{C}_{\text{SL}})$$

where C<sub>LT</sub> is the litter derived portion of the SOC, C<sub>S</sub> the C in the sample, C<sub>SL</sub> is the C of the soil without *Bouteloua* litter and C<sub>BG</sub> is the C of the *Bouteloua* litter. This also holds for the chloroform fumigation extracts. The C content of the fumigated extracts (C<sub>f</sub>) is the sum of the C content of the non fumigated (control) extracts (C<sub>c</sub>) and the additional extracted C from cell lysis by chloroform (chloroform-labile C; C<sub>b</sub>):

$$C_f = C_c + C_b$$

Accordingly, the  $\delta^{13}\text{C}$  of chloroform fumigation extracts is:

$$\delta^{13}\text{C}_f \cdot C_f = \delta^{13}\text{C}_c \cdot C_c + \delta^{13}\text{C}_b \cdot C_b$$

*Litterbags in different grassland types.* To study grassland litter decomposition, a field experiment with litterbags was carried out. PVC rings (6 cm x 10 cm diameter) were fitted with hose clamps to fix screen (mesh size = 1 mm) on the open sides. They were placed at a site with relict perennial bunchgrasses and at a site with restored perennial grassland, continuously tilled, bare soil, and annual grassland. In total 130 litterbags were placed in the field on Oct. 31, 2002, right before the first fall rain. Litterbags were sampled on three different dates during the growing season. Sampling was done with five replicates. Litterbags were filled with 6 g of aboveground litter from their respective sites, either *Nassella pulchra* or annuals, e.g., litterbags for relict or restored perennial grasslands were filled with *Nassella pulchra* litter. Aluminum

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content was also determined for correcting the results for contaminating soil (Potthoff and Loftfield 1998).

## Results

*Comparison of soil C, microbes, and plant roots in grassland soils with different management histories.* Grassland soils had higher total C, microbial biomass C, and respiration the uppermost layer (0-15 cm depth), and greater CO<sub>2</sub> efflux from the soil surface than in the tilled soil, as described previously in last year's annual report (Jackson et al. 2003). The two grasslands were similar in terms of these soil surface properties. Few differences in soil biological or chemical properties occurred below 15-cm depth, except that at lower depths, the CO<sub>2</sub> concentration in the soil atmosphere was lower in the tilled plots, possibly from reduced production of CO<sub>2</sub> due to the lack of root respiration. Root length near perennial bunchgrasses was lower at the surface and greater at lower depths than in the annual grass-dominated areas; a similar but less pronounced trend was observed for root biomass. The similarity in microbiological properties in soil layers below 15-cm depth suggest that deeper microbiota rely on more recalcitrant C sources and are little affected by plant removal compared to the surface layer, even after six years without plants present (Potthoff et al. 2005).

Further sampling of CO<sub>2</sub> concentration in the soil atmosphere during the spring of 2003 confirmed the earlier results that activity did not differ significantly between soils dominated by annuals vs. by perennial bunchgrasses in the restored perennial grassland. For five sampling dates from March through June, there was no consistent difference at any of the four depths between the grasslands, although perennials tended to have higher CO<sub>2</sub> concentration below 20-cm depth (data not shown). The CO<sub>2</sub> concentration in the soil atmosphere of the tilled soil was typically about half that of the grasslands from March through early May, but differences decreased after soil began to dry out in late May. More detailed analysis of soil drying profiles may reveal relationships between CO<sub>2</sub> production and moisture (Hopmans et al. 2000). Soil CO<sub>2</sub> emission, as determined in the closed chambers, showed a similar pattern, i.e., similar rates in the two grassland soils, and lower emissions from the tilled soil until late May when soil moisture decreased (data not shown).

Although ecosystems with deep roots are known to contain more soil C at depth in the soil profile (Boutton et al. 1999), the rate of deep C accumulation may be slow, and limited by environmental constraints, such as water-holding capacity. Native perennial bunchgrasses in the restored grassland had only slightly deeper root distribution than annuals, indicating slow potential for C sequestration deep in the soil profile. The restoration process, however, did not result in net C loss, despite the necessity of tillage to reduce the annual seedbank before planting perennial grasses. At four years after planting native grasses, total C, soil microbial biomass and activity was nearly the same as the former conditions represented by annual grassland, suggesting high resilience to the temporary disturbance caused by prior tillage. The loss of C due to temporary tillage may be eventually compensated if native perennial bunchgrasses ultimately cause greater C accumulation than non-native annual grassland. Increased biodiversity may be another asset of restoration to native perennial grassland (Steenwerth 2003).

*Fungal markers in grassland soils with different management histories.* The microbial biomass of temperate grassland soils can be rich in fungi (Ruzicka et al. 2000). Fungi are effective in decomposing litter of poor quality with associated impacts on C flow and fertility in soil. Fungal

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distribution in the soil profile may change in relation to root vs. shoot litter availability. Using the same soil collected in the previous experiment, depth differences between soils with different management histories were compared using phospholipid fatty acid (PLFA) analysis, with emphasis on the fungal biomarkers 18:3 $\omega$ 6c (Vestal and White 1989; Bååth and Anderson 2003), 18:2 $\omega$ 6 (Federle 1986; Zelles 1997; Bååth and Anderson 2003), and 18:1 $\omega$ 9c (Schutter and Dick 2001; Bååth and Anderson 2003), and ergosterol content.

**Table 1.** Significant correlations ( $P < 0.05$ ) for (a) total amounts of soil microbial biomass as determined by chloroform fumigation (MBC), total PLFA (PLFA), fungal PLFA markers, their sum and ergosterol and for (b) ratios of PLFA fungal markers to MBC, their sum and ergosterol.

(a)						
	MBC	PLFA	18:3 $\omega$ 6c	18:2 $\omega$ 6	18:1 $\omega$ 9c	sum
PLFA	0.92					
18:3 $\omega$ 6c	0.90	0.86				
18:2 $\omega$ 6	0.86	0.89	0.87			
18:1 $\omega$ 9c	0.92	0.94	0.92	0.97		
sum	0.91	0.93	0.92	0.98	1.00	
ergosterol	0.89	0.89	0.83	0.86	0.91	0.90

(b)				
	18:3 $\omega$ 6c	18:2 $\omega$ 6	18:1 $\omega$ 9c	sum
18:2 $\omega$ 6	0.53			
18:1 $\omega$ 9c	0.67	0.74		
sum	0.65	0.90	0.92	
ergosterol	0.33	0.53	0.78	0.69

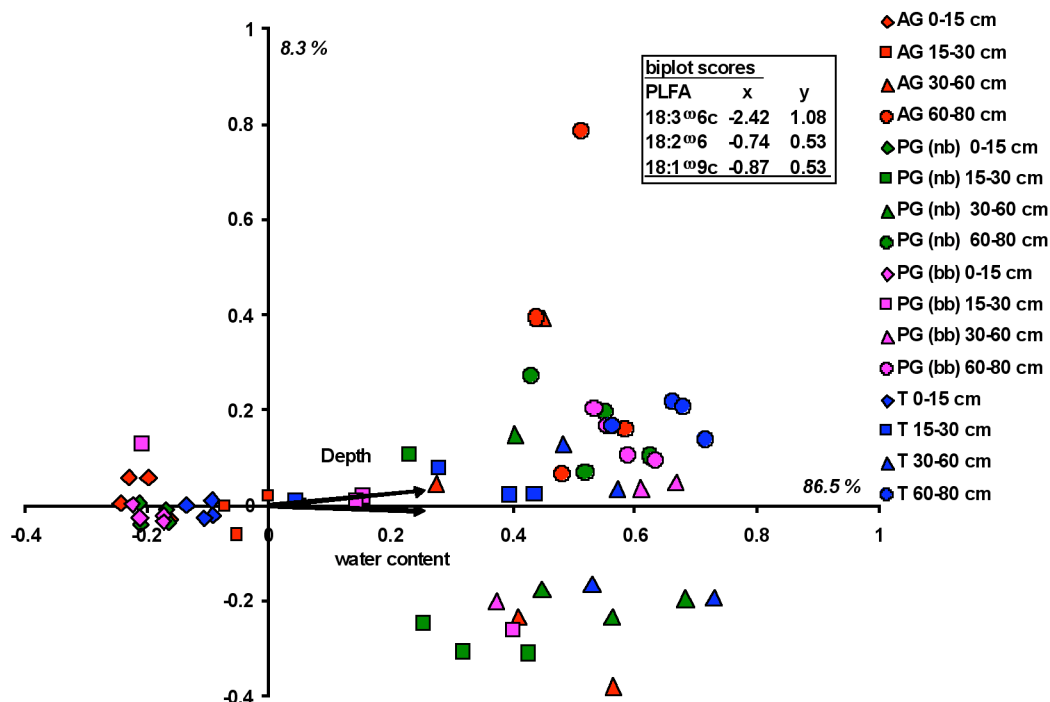
Soil depth was the strongest factor explaining differences in microbial community composition among grassland and tilled soil samples as detected by PLFA profiles, based on the CCA ordination (*fig. 1*). PLFA profiles were generally similar between management types, except for the surface soil. The fungal PLFA biomarkers showed strong biplot scores that were negatively related to soil depth in CCA. Specific ratios of each biomarker to microbial biomass C (chloroform-fumigation) showed that the fungal portion of the microbial community decreased with depth (*table 1*). The PLFA fungal marker 18:3 $\omega$ 6c was not detected in the tilled bare plot and not beneath 15 cm, suggesting that this PLFA belongs to fungi in the surface soil associated with aboveground litter or surface roots (*fig. 1*). Fungal biomarkers were well-correlated with ergosterol content, as were specific ratios of these biomarkers to microbial biomass C (*fig. 2*).

These results suggest that ergosterol is a general fungal marker covering a broad spectrum of species. Single PLFA biomarkers may be more specific and may indicate functional groups, for example, as primary colonizers of grass litter or associated with living roots. In combination, these fungal markers appear to provide effective tools to explore microbial community changes related to the fungal portion of soil microbial biomass, and its role in C processes in soil.

*Root effects on soil C dynamics and microbial communities.* Perennial bunchgrasses were expected to have substantial effects on soil C pools and microbial community composition in

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their rootzones, compared to nearby bare soil areas (Hook et al. 1994; Vinton and Burke 1995). Microbial biomass C and dissolved organic C were greater under perennial bunchgrasses in the relict perennial grassland and perennial grass agriculture sites when comparing seasonal means of bare vs. plant soil, but not in the restored perennial grassland or annual grassland. Microbial biomass C decreased from fall to spring in both bare and plant soils of some sites. Soil moisture, microbial respiration, and nitrate decreased from fall to spring in both bare and plant soils, while soil ammonium and net mineralizable N (plant only) increased.

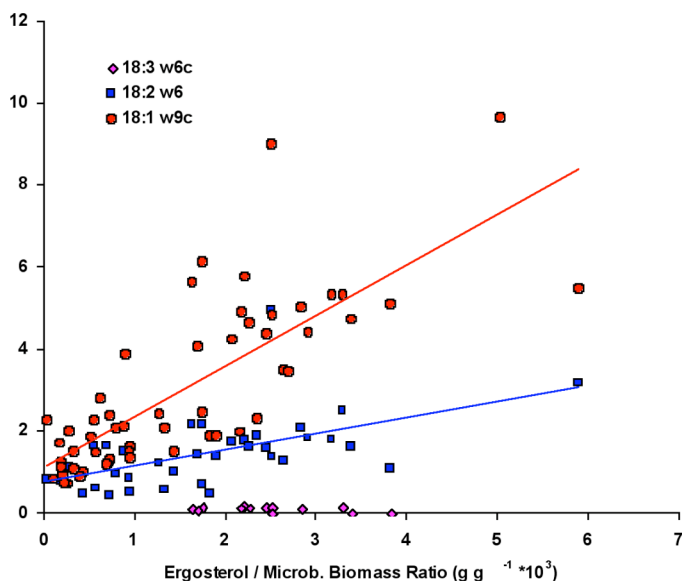


**Figure 1.** Multivariate statistical analysis (CCA) for PLFA extracted from grassland soil samples at four depths at the three neighboring grassland sites. Soil samples were taken in April, 2002. AG=Annual grassland. PG (nb)=Perennial grassland (near bunches). PG (bb)=Perennial grassland (between bunches), T=Tilled for 6 years with no plant cover. The biplot scores for three fungal PLFA biomarkers are shown.

Using multivariate analyses, PLFA profiles consistently differentiated by land use history in fall, winter, and spring. The cluster of annual grassland samples slightly overlapped with that of restored perennial grassland, showing less similarity than in the analysis of the entire profile that was described above. When each site was analyzed separately from other sites, PLFA profiles formed distinct clusters according to plant or bare soil in winter and spring. Also, within an individual site, PLFA profiles clustered by season. These results suggest that effects of perennial and annual grasses corresponding to increased plant growth in winter and spring or abiotic differences between bare and plant soils influenced microbial community composition. Individual PLFA that distinguish microbial communities under perennial bunchgrasses varied widely (*fig. 4*). Bulk soil properties and the individual plant may have a strong effect on soil microbial community composition.

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*Carbon flow using C<sub>4</sub> grass residue with and without legumes.* The effect of plant species composition on C flows was assessed in large field microcosms at one of the restored native perennial grasslands at the Hastings Reserve. Each contained a native perennial bunchgrass (*Nassella pulchra*), and received one of eight treatments with different plant species and litter combinations (see above). The treatments were chosen to compare different inputs for their effect on soil C flows through the microbial biomass to SOC, including the effect on plant productivity, which can ultimately increase C sequestration in soil. These are long-term processes, and the microcosms are large enough to sample repeatedly over a period of three years. The microcosms were installed in December 2002, and the last sampling to date was in April 2003, with further sampling planned for 2004-5.



**Figure 2.** Correlation of PLFA fungal markers to ergosterol, both expressed as relative amounts, i.e., as ratios to soil microbial biomass C using the chloroform fumigation extraction method. Soil samples from the three grassland types and four depths as described in Fig. 1.

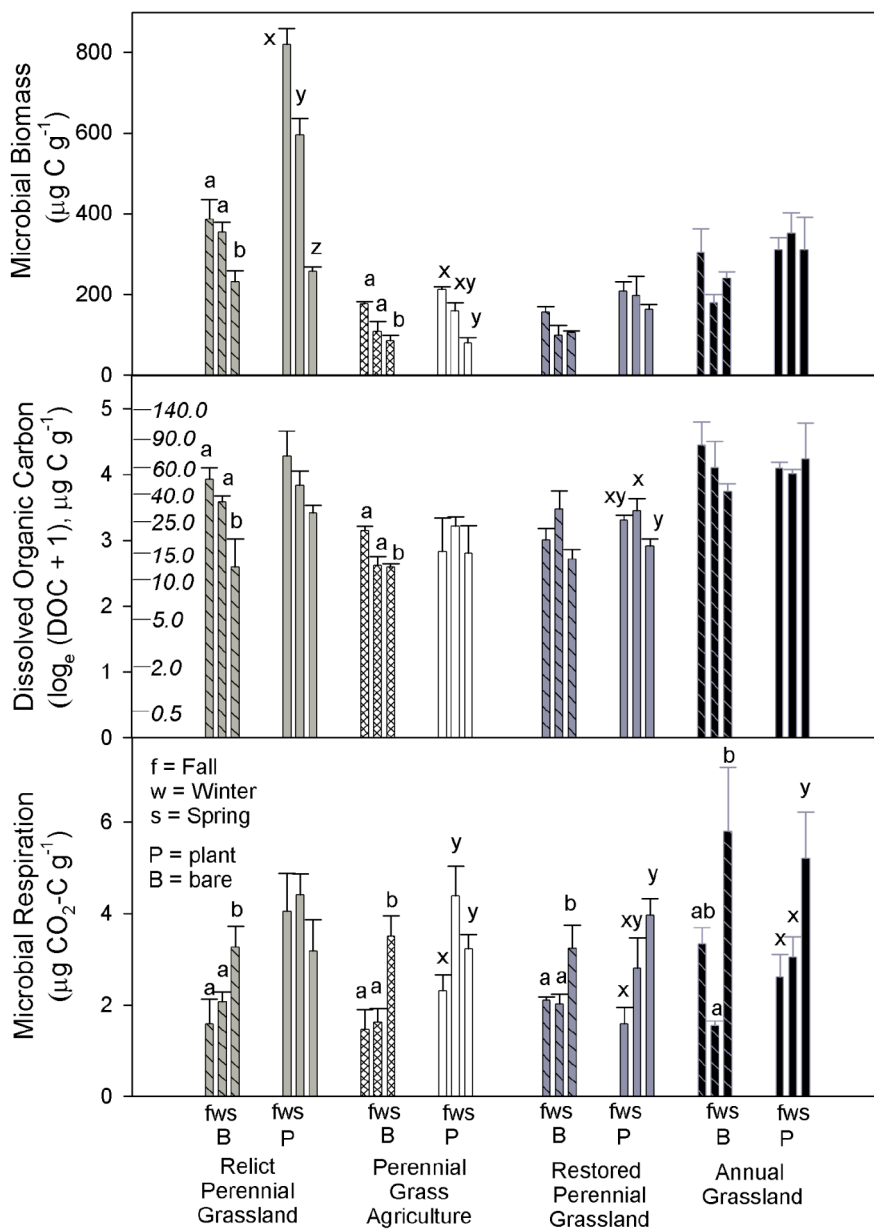
Since the litter of the C<sub>4</sub> bunchgrass, *Bouteloua gracilis*, has similar C/N ratio as the native perennial grass, *Nassella pulchra*, it can serve as an indicator of fates of litter of the native grass, which otherwise would be difficult to track, since the  $\delta^{13}\text{C}$  content of *Nassella pulchra* is similar to ambient soil values. The quantity of C<sub>4</sub> plant litter added to the field microcosms was almost twice that of the existing C<sub>3</sub> litter, to test if adding perennial bunchgrass litter could aid restoration efforts, especially with suppression of non-native annuals. To further address effects of plant species composition on soil C flows and on plant productivity, a native annual legume (*Lupinus bicolor*) and/or added phosphorus (P) was added in some treatments.

Microbial biomass C at 0-7.5 cm depth was not affected by litter or nutrient treatment after five months (fig. 5). There was rapid assimilation of C by microbial biomass from the *Bouteloua gracilis* C<sub>4</sub> litter, based on its  $^{13}\text{C}$  enrichment, but legumes and added P had no effect (fig. 6). The addition of this high C/N perennial grass litter, however, decreased aboveground productivity of annual vegetation in the cylinders, resulting in lower C assimilation by plants (fig. 7). Lupin treatments did increase plant aboveground biomass, but there was no effect of added phosphorus.



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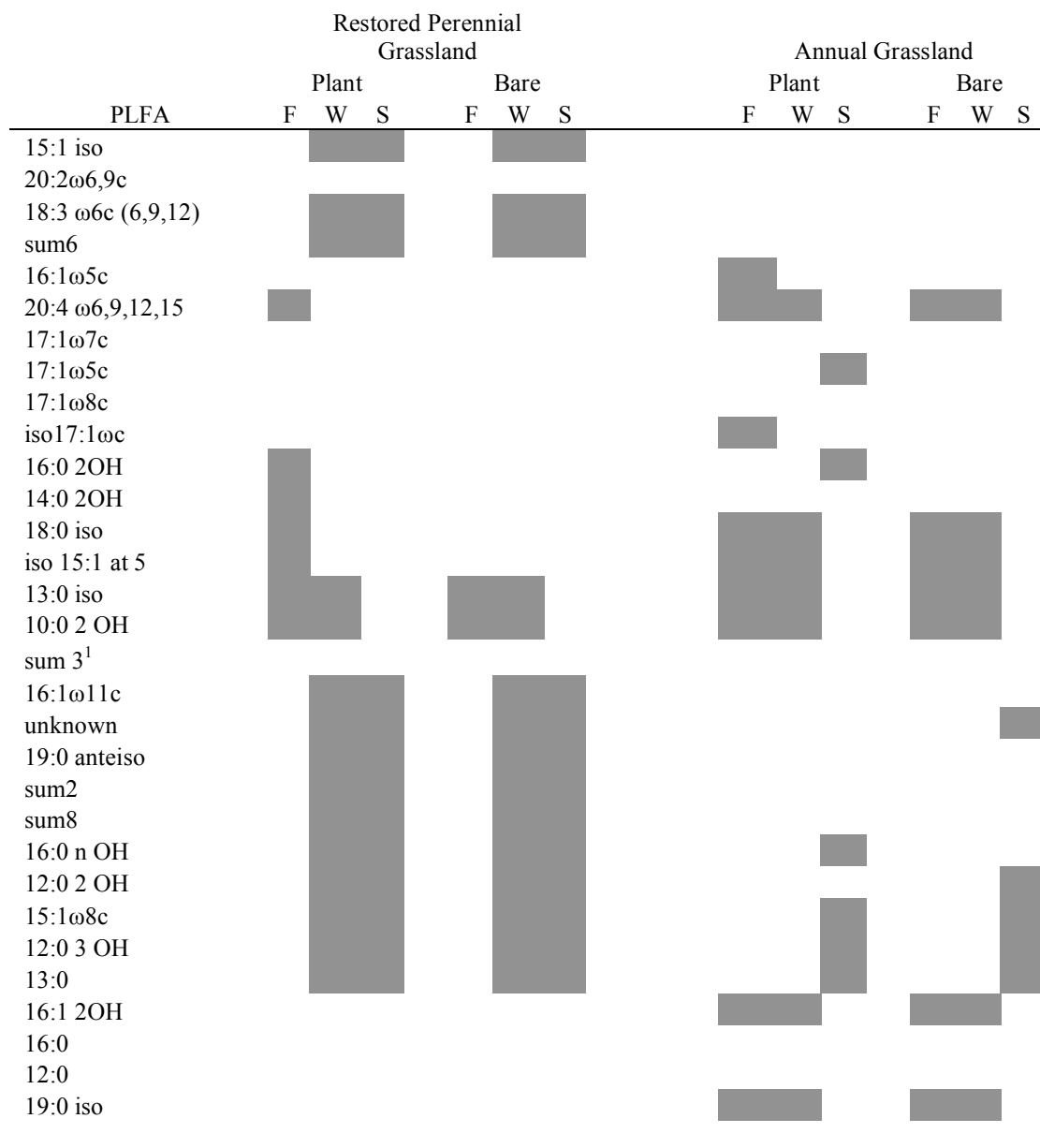
Overall, the initial results from this field microcosm study indicates more pronounced effects of litter quality on plant productivity than on microbial biomass, but it shows that turnover of litter C in the surface layer can be very rapid during the spring. Further work will indicate longer-term effects on soil C, as well as on productivity of annuals and perennials, which will be harvested for above- and belowground biomass distribution at the end of the experiment in 2005.



**Figure 3.** Soil microbial biomass C, dissolved organic carbon and microbial respiration for the four sites with different land management history on granitic and alluvial soils in Carmel Valley (0-12 cm). Italicized numbers along the y-axis indicate the concentrations of pools after back transformation of DOC to reach a normal distribution. Letters indicate significant differences between seasonal means of a given plant or bare treatment for that site. "a" and "b" correspond to 'bare' soils. "x" and "y" correspond to 'plant' soils. Groups of bars without letters are not significantly different from each other. Pairwise comparisons by LS Means and adjusted Bonferroni mean separation test ( $P \leq 0.01$ ).

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Fig 4(a)



<sup>1</sup>Sum 3 is an unresolved mixture of 12:0 aldehyde, unknown 10.928,14:0 3OH and 16:0 iso I.

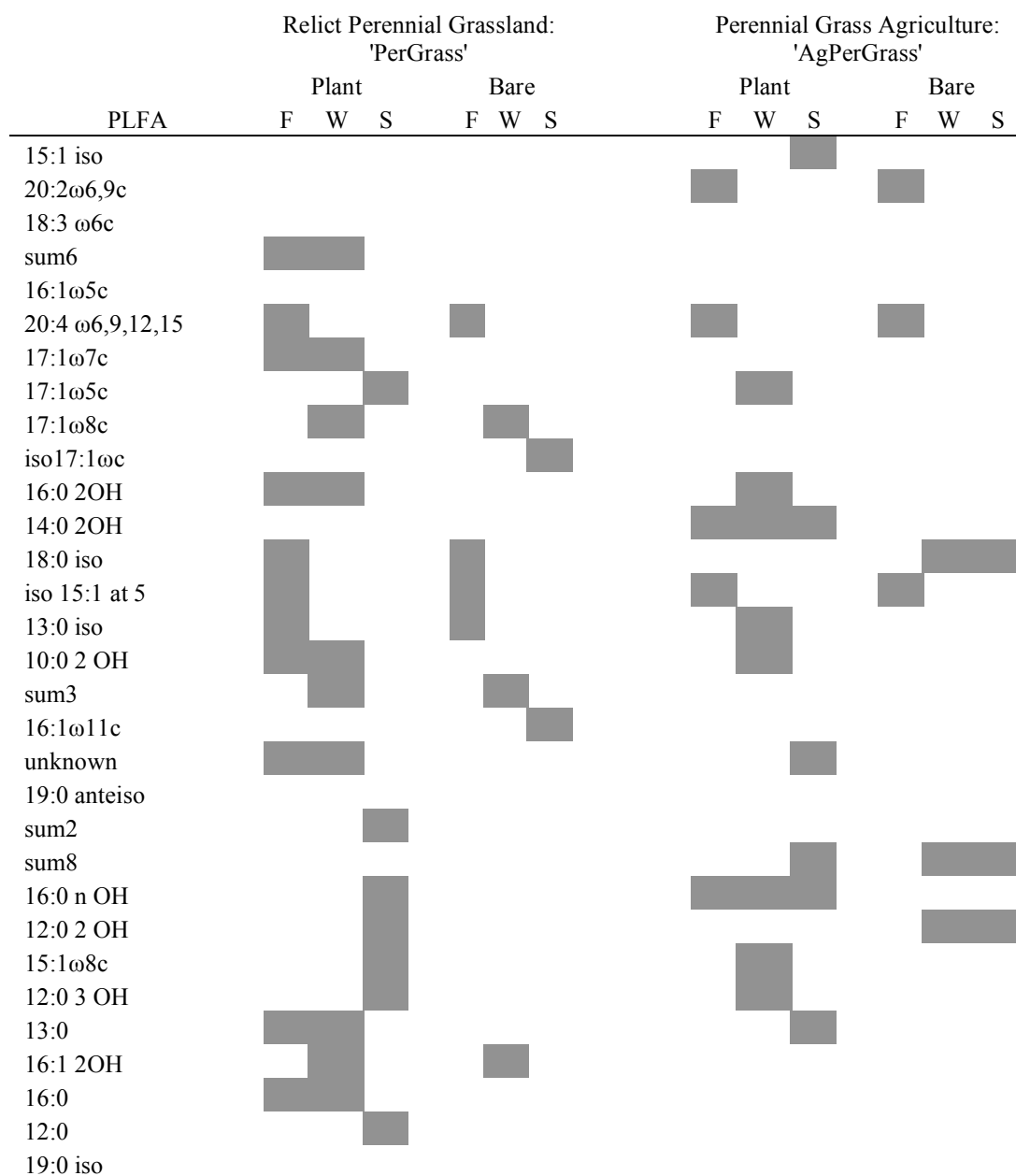
<sup>2</sup>Sum 2 is an unresolved mixture of 15:1 iso H, 13:0 3 OH, and 15:1 iso I.

<sup>3</sup>Sum 6 is an unresolved mixture of 18:0 anteiso and 18:2ω6,9c.

<sup>4</sup>Sum 8 is an unresolved mixture of unknown 18.756 and 19:1ω11c.

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**Fig 4(b)**



<sup>1</sup>Sum 3 is an unresolved mixture of 12:0 aldehyde, unknown 10.928,14:0 3OH and 16:0 iso I.

<sup>2</sup>Sum 2 is an unresolved mixture of 15:1 iso H, 13:0 3 OH, and 15:1 iso I.

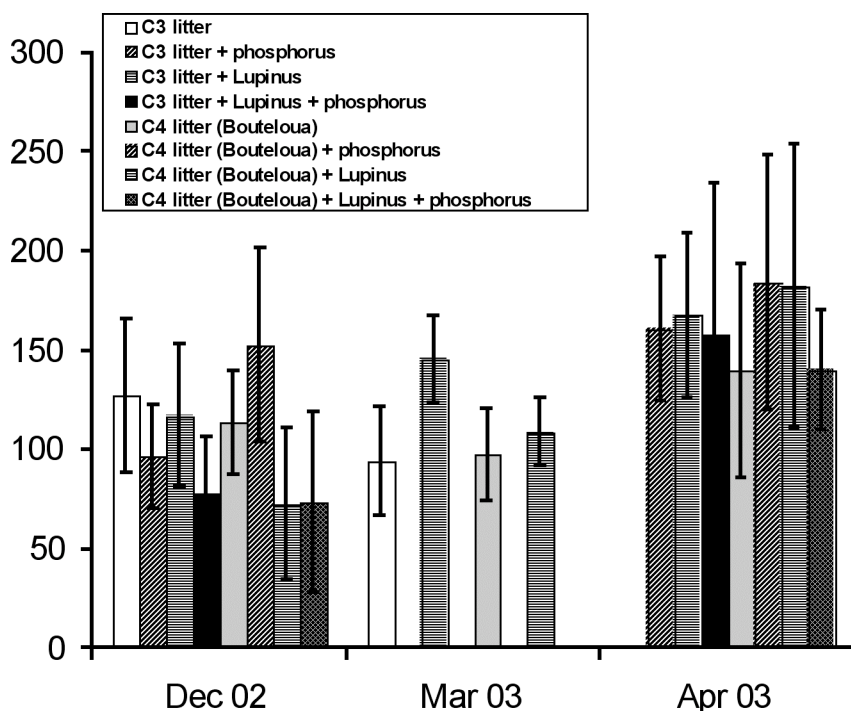
<sup>3</sup>Sum 6 is an unresolved mixture of 18:0 anteiso and 18:2ω6,9c.

<sup>4</sup>Sum 8 is an unresolved mixture of unknown 18.756 and 19:1ω11c.

**Figures 4a&b.** PLFA were from the CCA analyses in which each grassland land use type was analyzed separately to detect the effect of season and plant presence on microbial community composition. A grey square next to a PLFA marker indicates that it is positively associated with and has relatively greater abundance for the designated column. For example, 17:1ω5c is present in relatively higher abundance in 'AnnGrass' in the Spring in soils with plants. 'F'=fall, 'W'=winter, 'S'=spring. (a) restored perennial grassland and annual grassland which were adjacent at the Hastings Reserve; (b) relict perennial

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grassland and perennial grass agriculture at Rana Creek Ranch. All sites were on similar soil derived from granitic and schistose alluvium.

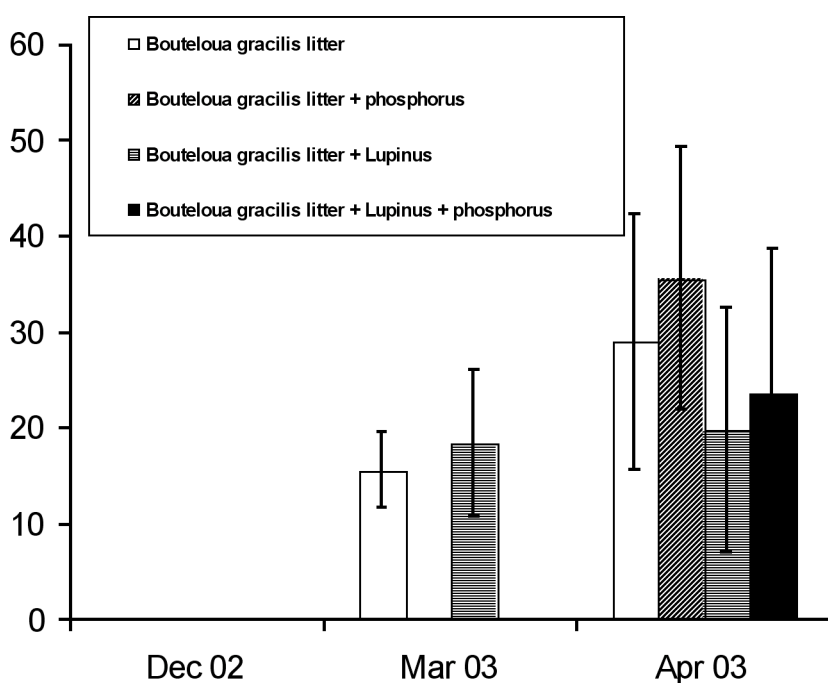


**Figure 5.** Soil microbial biomass C in the 0-7.5 cm layer of soil in the large cylinders installed in December 2002, with various litter, lupine, and phosphorus treatments. n=5 per treatment.

*Litterbags in different grassland types.* Rates of litter loss from litterbags varied according to the plant species composing the litter and site of placement. Litterbags containing annual grass litter in annual grassland lost 35% of their dry weight during the first three months (Oct. 31-Jan. 31), with no further change during the spring (data not shown). At the tilled site, only 15% of the litter was lost from Oct. 31 to June 23, indicating a much slower rate of decomposition in the disturbed, plant-free soil. Perennial grass litter was slower to decompose, with approximately 20% litter loss by Jan. 31, and a total of 30% litter loss by March 27, with little change by June 23. Rates of litter loss tended to be slower in the relict perennial grassland than the restored perennial grassland, but there were only minor differences due to position, e.g., between bunchgrasses, within a bunchgrass clump, or on a stake above the soil surface. In general, the loss of litter from the litterbags appears slow in relation to the typical disappearance of litter in California grasslands (Jackson et al. 1988), suggesting that litterbags had an influence on the abiotic or abiotic environment. One possibility is that the exclusion of fauna may result in slow decomposition. Further research on faunal effects may better explain rates of litter decomposition and C retention in soil. Although descriptive, the litterbag results indicate that a better understanding of plant species' effects on litter decomposition is necessary for understanding C flows in grassland.

## Discussion

*Root vs. shoot litter effects on soil C transformations.* Root inputs to soil from annual vs. perennial grasses resulted in only slight differences in soil C pools, soil CO<sub>2</sub> concentrations, and microbial community composition, based on PLFA profiles. After four years with native perennial grasses, root distribution with depth responded only slightly to the difference in grassland types by a significant interaction of depth and treatment in the analysis of variance, with no associated change in soil microbial composition. Over a longer period, turnover of perennial roots and production of root exudates may fuel microorganisms, possibly creating a gradual, slow process of increased microbial biomass and C-storage in lower layers of Central Coast grasslands after restoration of native perennial bunchgrasses.



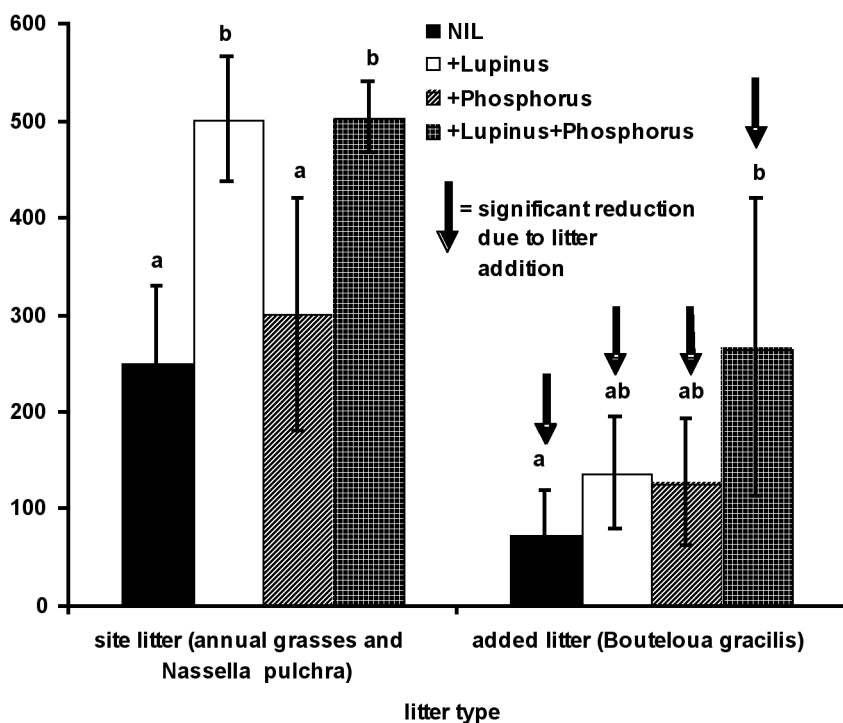
**Figure 6.** Percentage of litter-derived C in soil microbial biomass C in the 0-7.5 cm layer during the spring of 2003, after addition of C<sub>4</sub> *Bouteloua gracilis* litter in December, 2002, into large cylinders. n=5 per treatment.

The presence of roots of the native perennial bunchgrass, *Nassella pulchra*, in surface soil (0-12 cm) had variable effects on soil C availability and microbial composition, compared to nearby bare zones, in different types of grassland settings. Lack of a consistent plant root effect at all sites suggests that 1) roots cause slow changes in soil with time and were only pronounced with the much older plants at the relict perennial grassland, or in contrast with bare soils that had been tilled for decades at the perennial agriculture site, or 2) these typical measurements of C pools in bulk soil may not accurately portray effects of rhizodeposition. Although some differences in microbial community composition between plant and bare soils were observed, each site had a unique set of differences, so that no explicit PLFA biomarkers could be attributed to presence or absence of *Nassella pulchra*.

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In contrast, aboveground litter deposition has strong effects on processes in the surface (0-15 cm) soil layer, including microbial biomass, activity, and composition, since after two years of tillage, surface soil of the restored perennial grassland was similar to annual grassland four years later. Addition of C<sub>4</sub> litter showed rapid assimilation into the microbial biomass. Litterbags indicated that plant species composition and placement have a potentially large effect on C flows. Further study is needed to understand the unexpectedly large difference in soil responses to root vs. shoot inputs.

*Plant species composition, productivity, and C flows.* Plant species composition of California grasslands has a potentially larger effect on C transformations in the soil surface than deeper soil, at least during the short time frame of this study. The deposition of larger amounts of high C/N litter from perennial grasses resulted in similar amounts of soil microbial biomass as from lower C/N litter from the local C<sub>3</sub> vegetation. Decomposition rates of the perennial grass litter may have been slower, which was supported by the litterbag results. The rapid rate of assimilation of C by the microbial biomass from the added C<sub>4</sub> litter indicates the importance of the surface soil community in aboveground litter decomposition and C storage. Restriction of a fungal PLFA marker to the surface soil supports the idea that belowground biodiversity may also be important for these processes.



**Figure 7.** Estimated aboveground biomass of annual vegetation in the cylinders at the end of the growing season on May, 30, 2003, using a visual ranking that was correlated with clipped biomass on adjacent plots.

The addition of high C/N perennial grass litter had a negative effect on plant productivity in the first year after litter addition which, if sustained, could limit total C inputs into the soil. Sampling in 2004-5 will indicate changes with time and with higher P and legume inputs. Also,

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perennials are being evaluated for tiller number, and will be harvested at the end of the study to detect biomass and root distribution differences.

*Management implications.* These results indicate that highly manipulative procedures that employ repeated tillage and herbicide applications to reduce non-native grasses appear worthwhile for the restoration of native perennial grasses because the microbiota have a high resilience to return to their former performance after bunchgrasses are established for several years on cultivated sites. Also, microbial composition throughout the profile was generally similar in the annual and restored perennial grasslands, although more detailed seasonal analysis showed less similarity for the 0-12 cm layer. The restored perennial grassland was more similar in terms of C pools and microbial community composition to annual grassland, than to relict perennial grassland.

On this soil type, restoration of native perennial grassland did not result in greater C accumulation in soil or biomass after four years had passed. The major short-term incentive for the restoration effort would be to increased species richness of native plant species (Steenwerth 2003; Potthoff et al. 2005). Further sampling will indicate if added organic matter and planting of legumes during the early stages of the restoration process may promote greater soil microbial biomass and C sequestration in the surface layer.

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