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Soil microbial community composition as affected by restoration practices in California grassland

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Abstract

Agricultural practices have strong impacts on soil microbes including both the indices related to biomass and activity as well as those related to community composition. In a grassland restoration project in California, where native perennial bunchgrasses were introduced into non-native annual grassland after a period of intensive tillage, weeding, and herbicide use to reduce the annual seed bank, microbial community composition was investigated. Three treatments were compared: annual grassland, bare soil fallow, and restored perennial grassland. Soil profiles down to 80 cm in depth were investigated in four separate layers (0–15, 15–30, 30–60, and 60–80 cm) using both phospholipid ester-linked fatty acid (PLFAs) and ergosterol as biomarkers in addition to microbial biomass C by fumigation extraction. PLFA fingerprinting showed much stronger differences between the tilled bare fallow treatment vs. grasslands, compared to fewer differences between restored perennial grassland and annual grassland. The presence or absence of plants over several years clearly distinguished microbial communities. Microbial communities in lower soil layers were little affected by management practices. Regardless of treatment, soil depth caused a strong gradient of changing habitat conditions, which was reflected in Canonical Correspondence Analysis of PLFAs. Fungal organisms were associated with the presence of plants and/or litter since the total amount and the relative proportion of fungal markers were reduced in the tilled bare fallow and in lower layers of the grassland treatments. Total PLFA and soil microbial biomass were highly correlated, and fungal PLFA biomarkers showed strong correlations to ergosterol content. In conclusion, microbial communities are resilient to the grassland restoration process, but do not reflect the change in plant species composition that occurred after planting native bunchgrasses.

Keywords: PLFA; Ergosterol; Soil microbial biomass; Soil depth; Plant removal; Agricultural inputs

1. Introduction

Shifts in microbial species composition, especially after land use conversion and changes in vegetation as attributes of restoration, may be associated with changes in ecosystem function (Conrad, 1996; Copley, 2000; Steenwerth et al., 2003). Examples for links between microbial community composition and soil processes are given by Cavigelli and Robertson (2000) for the process of denitrification and by Balser and Firestone (2005) for the process of N mineralization in soils.

Little is known about the impact of restoration practices on soil microbial communities. Restoration in the US mainly focuses on the creation of plant communities that closely resemble those of undisturbed native vegetation prior to European settlement (Zedler, 2001; Walker and del Moral, 2003). Today native California grassland communities are limited to rare relict sites while most of the area is dominated by non-native annual grasses from the Mediterranean Basin

(Huenneke, 1989). The non-native annuals were able to outcompete the native perennial grasses after overgrazing and drought (Burcham, 1957; Jackson, 1985). A successful method for native grassland restoration has been to use agricultural techniques such as tillage and herbicide application for 2–3 years to control exotic annuals before seeding with native perennials (Stromberg and Kephart, 1996). Such practices have a strong impact on soil biology, biochemistry and microbial communities (Doran, 1980; Follett and Schimel, 1989; Aon et al., 2001; Steenwerth et al., 2003).

Restoration of old-field exotic annual grasslands to native perennial grasslands using agricultural inputs can affect soil microbial activity, CO_2 concentrations in soil, and root distribution in the soil profile, as well as surface CO_2 efflux (Potthoff et al., 2005a). In addition to changes in soil respiration and chemical soil characteristics, shifts in microbial communities can occur in the surface soil in response to specific factors involved in the restoration process (e.g., Calderón et al., 2001; Steenwerth et al., 2003). Grassland restoration may also be associated with shifts in microbial community composition at greater depths in the soil profile because perennial bunchgrasses support higher root biomass at depth than annual grassland species (Holmes and Rice, 1996; Potthoff et al., 2005a).

To maintain consistency with previous studies (Steenwerth et al., 2003), microbial community composition and diversity were described by phospholipid ester-linked fatty acid (PLFA) analysis. PLFA profiles were analyzed by multivariate statistics, an approach that generates fingerprints of the microbial community. The microbial fingerprints vary with the number, type and amount of detected PLFAs in the soil (Kent and Coker, 1992). Single PLFA also serve as biomarkers for tracking the presence and relative abundance of special functional groups (Zelles, 1999). These can be compared with other biomarkers to validate shifts in community composition. For example, both fungal PLFA biomarkers (Zelles, 1999) and ergosterol (Djajakirana et al., 1996) may differ with management, as fungal species are known to be the first colonizers on grass litter with wide C/N ratios (Bowen and Harper, 1990), but they are also very sensitive to dry conditions (Frey et al., 1999).

In order to investigate potential changes in microbial community composition in a restoration project in coastal California grassland (Stromberg and Kephart, 1996; Stromberg et al., 2002; Potthoff et al., 2005a), this study addressed the following questions: (1) What changes in microbial communities and fungal markers occur during native grassland restoration? (2) Do shifts in plant communities affect microbial communities in lower layers that were not physically disturbed by tillage and herbicide application?

2. Material and methods

2.1. Site and treatments

Our investigations took place in April 2002, during the season of peak biological activity, at the UC Hastings Natural History Reservation in the foothills of the Santa Lucia Mountains in Upper Carmel Valley (Longitude: 121° 31′ 0′ N; Latitude 36° 12′ 30′ W). The study site was on a Sheridan coarse sandy loam soil (coarse-loamy, mixed, thermic Pachic Haploxerolls; Cook, 1978) located on a level area that had been farmed between 1865 and 1937. Physical and chemical soil properties are listed by Potthoff et al. (2005a). The climate of this region is Mediterranean, with small annual amplitudes in daily mean temperatures (15.5 °C in summer and 13.0 °C in winter; Cook, 1978). Mean annual precipitation is 540 mm and typically occurs from September to May.

Three different plant management treatments representing various stages of native grassland restoration (Potthoff et al., 2005a) were investigated in this study:

(1) Tilled bare soil from former old field annual grassland that was plant-free for 6 years using tillage and Roundup herbicide (glyphosate, Grass/Weed Killer, Ortho, Columbus, OH).
(2) Old field annual grassland, left fallow 65 years ago.
(3) Four-year-old restored perennial grassland that was created after tilling annual grassland and planting with native bunchgrasses (*Nassella pulchra* [Agrostideae tribe], *Elymus glaucus* [Hordeae tribe] and *Hordeum brachyantherum ssp. californicum* [Hordeae tribe]):
    (a) Sampling zone between bunches of *Nassella pulchra*, at least 20 cm away from a bunchgrass ('between bunchgrass').
    (b) Sampling zone very close to *Nassella pulchra* plants at the perimeter of the bunchgrass crown ('near bunchgrass').

2.2. Sampling and analysis

Soil sampling was completed during an intensive 3-day campaign (Potthoff et al., 2005a). Every treatment and depth combination was sampled with four replicates (two samples in one of two blocks) including the determination of total C content and soil microbial biomass C (SMB-C). PLFA analysis as well as ergosterol content was investigated to obtain indicators on the diversity and composition of microbial communities supported in the treatments. Soil measurements were taken in four soil layers (0–15, 15–30, 30–60 and 60–80 cm).

From the sides of two soil pits per treatment per sampling block, soil blocks (15 × 15 cm × height of the layer in size) were removed from the midpoint of each sampling depth. The soil was gently mixed and subsampled for analysis.

The protocol for PLFA analysis followed Bossio and Scow (1998). Total lipids were extracted from moist soil samples (8 g dry soil) using a chloroform–methanol extraction (Bligh and Dyer, 1959) modified to incorporate a 0.05 M phosphate buffer. The PLFAs were purified from the lipids

extracts, quantified, and identified using a Hewlett Packard 6890 Gas Chromatograph fitted with a 25 m Ultra 2 (5% phenyl)-methylpolysiloxane column (J & W Scientific, Folsom, CA). Individual PLFAs were identified using bacterial fatty acid standards and MIDI peak identification software (Microbial ID, Newark DE). The fatty acids were quantified by comparing the individual PLFA peak areas with that of the internal standard 19:0. Fatty acid terminology utilizes 'A:BωC' in the definitions given by Steenwerth et al. (2003) and Joergensen and Potthoff (2005). PLFAs were expressed in nmol PLFA g^{-1} dry soil.

PLFAs were grouped into bacterial (actinomycetes, Gram$^+$, Gram$^-$), fungal and unspecific origins following Kroppenstedt (1985), Federle (1986), O'Leary and Wilkinson (1988), Vestal and White (1989), Olsson et al. (1995), Zelles (1997), and Bossio and Scow (1998). For their own specific biomarkers in PLFA actinomycetes were listed apart from Gram$^+$ bacteria. A detailed grouping is given in Table 1.

For comparison of means the multivariate Tukey-HSD (Honest Significant Difference) test was applied to the data grouped by the factors Treatment and Depth (Statistica; Statsoft, Tulsa, OK). The level for significant differences was $P<0.05$. Statistics were applied to four replications for each treatment.

In addition, comparisons among PLFA profiles (taken as fingerprints of the microbial community) and relationships between PLFA profiles and soil characteristics were analyzed by Canonical Correspondence Analysis (CCA, Canoco 4.0; Microcomputer Power, Ithaca, NY). PLFAs that were detected in less than 10% of the samples were excluded from further analysis. Positions of samples along the axes are determined by loading scores, which describe the relative importance of a variable along the ordination axis. Soil characteristics are indicated by vectors on the biplots. The position of the vector indicates the variable's direction of increase, and comparison of the location of samples and vectors indicate the relationship between samples and environmental variables (e.g., samples plotted in the direction of increase of the environmental variable are enriched in this variable).

3. Results

3.1. Soil microbial biomass

As background information, after 6 years without vegetation, SMB-C, as determined by chloroform fumigation extraction, decreased significantly in the tilled bare plots (94 µg g^{-1} dry soil) compared to the old-field annual grass-

Table 1
Total PLFA, PLFAs derived from bacteria, fungi, or from unspecific origin detected in annual grassland ('ag'), restored perennial grassland ('pg') with near bunchgrass (nb) and between bunchgrass' (bb) zones, and tilled bare soil (ts) treatments. Bacterial PLFAs are separated as actinomycetes, Gram$^+$, and Gram$^-$. Actinomycetes are listed apart from other Gram$^+$ bacteria. PLFAs with frequencies less than 10% in all samples are not listed. Absolute PLFA concentrations (abs.) are given in nmol g^{-1}. The relative porportions of PLFAs from specific groups (rel.) is given in % of total PLFA ($n=4$, statistical grouping by Tukey HSD test; different letters indicate different groups)

Depth (cm)	Treat.	Total PLFA	All bacteria[a]		Actinomycetes[b]		Gram$^+$[c]		Gram$^-$[d]		Fungi[e]		Unspecific[f]	
		Abs.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.
0–15	ag	36.29b	16.08b	44.3	3.51b	9.7ab	7.17b	19.8a	4.96b	13.7	11.45b	31.5b	8.76b	24.2a
	pg (bb)	31.76b	14.81b	46.6	3.04b	9.6a	6.85b	21.6b	4.57b	14.4	9.39b	29.6b	7.56b	23.8a
	pg (nb)	29.62b	14.03b	47.4	2.93b	9.9ab	6.51b	22.0b	4.25b	14.3	8.41b	28.4b	7.18b	24.2a
	ts	15.34a	7.51a	50.0	1.71a	11.1b	3.50a	22.8b	2.20a	14.3	3.73a	24.3a	4.10a	26.7b
15–30	ag	14.73	7.03	47.7	1.81	12.3	3.05	20.7a	2.07	14.0b	3.73	25.3	3.97	26.9
	pg (bb)	15.32	7.48	48.8	1.89	12.3	3.39	22.1ab	2.06	13.4b	4.00	26.1	3.84	25.1
	pg (nb)	8.31	4.62	55.6	1.15	13.8	2.32	27.9b	1.10	13.2b	1.61	19.4	2.08	25.0
	ts	8.32	4.22	50.7	1.24	14.9	1.98	23.8ab	0.97	11.6a	1.78	21.4	2.32	27.9
30–60	ag	6.83	3.66	53.6	1.03	15.1	1.68	24.6	0.94	13.8	1.35b	19.8	1.82	26.6
	pg (bb)	3.58	2.11	58.9	0.72	20.1	1.00	27.9	0.39	10.9	0.58a	16.2	0.89	24.9
	pg (nb)	4.60	2.73	59.3	0.83	18.0	1.37	29.8	0.53	11.5	0.70ab	15.2	1.17	25.4
	ts	4.85	2.69	55.5	0.90	18.5	1.21	24.9	0.58	11.9	0.77ab	15.9	1.39	28.7
60–80	ag	4.01	2.14	53.4	0.76	18.9	1.02	25.4	0.36	9.0	0.72	17.9	1.15	28.7ab
	pg (bb)	2.18	1.20	55.0	0.37	17.0	0.66	30.3	0.18	8.2	0.34	15.6	0.64	29.3ab
	pg (nb)	3.00	1.67	55.7	0.50	16.7	0.92	30.7	0.24	8.0	0.51	17.0	0.82	27.3a
	ts	2.37	1.30	54.8	0.46	19.4	0.65	27.4	0.19	8.0	0.35	14.8	0.72	30.4b

[a]Sum of PLFA listed as actinomycetes, Gram$^+$ and Gram$^-$ + i15:1 (Federle, 1986; Zelles, 1997; Bossio and Scow, 1998).
[b]Sum of i17:1, 10Me16:0, 10Me17:0, and 10Me18:0 (Kroppenstedt, 1985; O'Leary and Wilkinson, 1988; Vestal and White, 1989).
[c]Gram$^+$ bacteria excluding acinomycetes; sum of 12:1, i14:0, a16:0, i15:0, a15:0, i16:0, i17:0, a17:0 (Federle, 1986; Zelles, 1997).
[d]Sum of 16:1ω7t, 16:1ω7c, 17:1ω9c, cy17:0, and cy19:0 (Federle, 1986; O'Leary and Wilkinson, 1988; Zelles, 1997).
[e]Sum of 16:1ω5c, 18:3ω6, 9,12c, 18:2ω6,9c, 18:1ω9c (Federle, 1986; O'Leary and Wilkinson, 1988; Vestal and White, 1989; Olsson et al., 1995; Zelles, 1997).
[f]Sum of unspecific PLFAs 12:0, 13:0 3OH, 14:0, 14:0 3OH, 15:0, 15:0 3OH, 16:0, 17:0, i17:1ω5c, 16: 2OH, 16:1ω11c, 18:0, cy19:0ω10c, 20:2ω6,9c, 20:0.

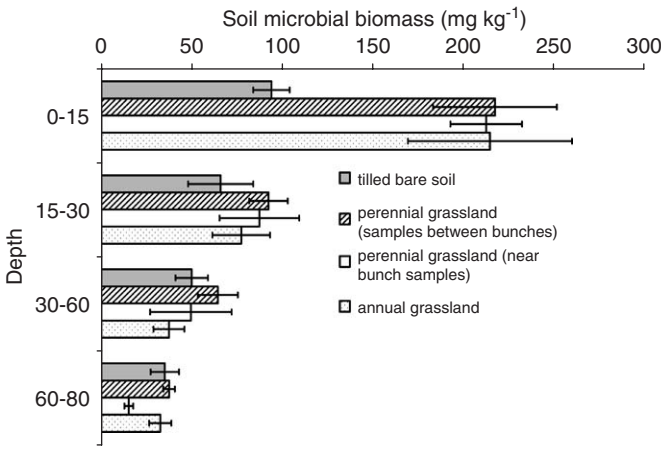

Fig. 1. Soil microbial biomass C in 0–80 cm soil profiles in annual grassland, restored perennial grassland and tilled bare soil at Hastings Reserve in Carmel Valley. Restored perennial grassland is represented by two sampling zones, near the bunches (nb) and between the bunches (bb) ($n = 4$, standard error bars, statistical grouping by Tukey HSD).

land (215 $\mu g\,g^{-1}$ dry soil) which occupied the site for >60 years before the tilled treatment began in the upper 15 cm of the soil profiles (Fig. 1, Potthoff et al., 2005a). In the surface of the restored perennial grassland, SMB-C recovered to similar values as in the old-field annual grassland 4 years after seeding of bunchgrasses. In tilled bare soil, SMB-C below 15 cm was not different from the grasslands. SMB-C tended to decrease with depth for all treatments (Fig. 1). Total soil C was significantly reduced from about 10 $g\,kg^{-1}$ in the untreated annual grassland to 6.8 $g\,kg^{-1}$ in the tilled bare soil treatment (Potthoff et al., 2005a). The ratio of SMB-C to total soil C was two to three times higher in the upper layer of the annual and perennial grasslands compared to both the tilled bare plot and all samples from deeper layers. Total PLFA, a second measure of soil microbial biomass, was closely related to SMB-C ($r = 0.92$). Both SMB-C and total PLFA were significantly correlated with total soil C ($n = 64$, $r = 0.77$ and 0.79, respectively).

3.2. Microbial community composition by canonical correspondence analysis

In total, 38 different PLFAs were detected in the soil samples from all treatments. Twenty-four of these could be classified as indicators for special microbial groups in the community (see Material and Methods). Fifteen PLFAs were not linked to specific soil microbial organisms (Table 1). The total number of PLFAs decreased from 38 in the surface layers of all treatments, to 29 and 25 in the 30–60 and 60–80 cm layer, respectively.

In the CCA biplot, axis 1 explained 86.5% of the variation, and axis 2 explained only 8.3% of the variation. Samples clustered by depth, but not clearly by treatment, except in the surface layer (Fig. 2). Soil depth and gravimetric water content were closely correlated with axis 1, and thus explained the distribution of samples along the first axis (forward selection, Monte Carlo, $P < 0.05$). Water content in the 0–15 cm layer was $5 \pm 1\%$. In all treatments, gravimetric water content increased with depth and was approximately $10 \pm 2\%$ in the 60–80 cm layer.

Other soil characteristics that were incorporated in the CCA analysis of the microbial community were not significant in explaining the variation in PLFA data. These variables included total C, total N, pH, bulk density, respiration, and root biomass (Potthoff et al., 2005a). Treatment (i.e., tilled bare soil, annual grassland, 'between bunchgrass', and 'near bunchgrass') also was not significant.

In the CCA of all treatments and depths (Fig. 2), all 0–15 cm samples from annual and perennial grassland soils clustered at –0.2 on axis 1. A second group of samples from 0 to 15 cm of the tilled bare plots and 15–30 cm of the annual grassland clustered to the right of the grassland surface soils. In contrast, all other samples showed much stronger variation along both axes. Samples of the 15–30 cm layer covered a wide range along axis 1 (−0.2 to 0.4). No samples collected lower than 30 cm ordinated left of 0.4 on axis 1. Samples from the lowest soil depth (60–80 cm) in all treatments clustered together between 0.5 and 0.7 along axis 1. Variation of the two upper layers along axis 2 was relatively low.

Certain PLFAs were strongly associated with axis 1. The most positive loading scores for axis 1, i.e., with increasing depth and moisture content, were found for $Gram^-$ a16:0 (4.28), $Gram^+$ 12:1 (3.40), and nonspecific 16:1 2OH (3.06). Biomarkers such as bacterial i15:1 (−2.55), fungal 18:3ω6,9,12c (−2.42), and non-specific 20:2ω6,9c (−2.03) were associated with the surface layer.

Another set of CCA were calculated to compare microbial communities of each layer across treatments. With the exception of the upper layer (0–15 cm), no clustering of microbial communities due to treatment or soil characteristics was observed (data not shown). In the CCA of the surface layer only, axis 1 explained 83.4% of sample variation, and axis 2 explained 6.0% (Fig. 3). Here, microbial communities from the tilled bare plot formed a distinct group that clustered separately (between 0.1 and 0.2 on axis 1) from microbial communities of both annual and perennial grassland soils. Also, soil microbial communities from the restored perennial plot (*Nassella pulchra* 'between bunchgrass' and 'near bunchgrass' treatments) tended to cluster independently from both the annual grassland and the tilled bare soil treatment. SOC and SMB-C were significant in explaining variation in soil microbial communities in the surface layer (Monte Carlo, $P < 0.05$), and were negatively correlated with axis 1.

3.3. Effects of grassland restoration on biomarkers of the soil microbial community

When the concentrations of specific PLFA were compared across field treatments, significant differences between treatments were detected in the surface layer, but generally not in the subsurface layers (Table 1). In comparison to the old-field annual grassland and the 'near

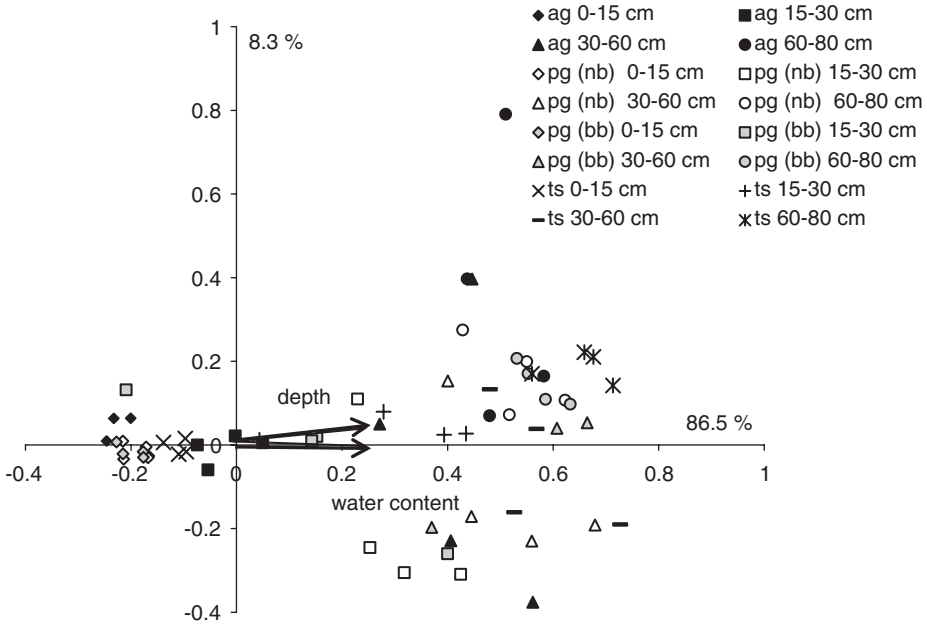

Fig. 2. CCA ordination of PLFAs extracted from 0 to 80 cm soil profiles in annual grassland (ag), in restored perennial grassland (pg), and tilled bare soil (ts) at Hastings Reserve in Carmel Valley, California. Perennial grassland was sampled in the 'near bunchgrass' (nb) and in 'between bunchgrass' (bb) zones. Plant cover in the 'between bunchgrass' zone consisted of annual grasses ($n = 4$). Significant factors for variation extracted from environmental data (soil depth and moisture) are given as vectors.

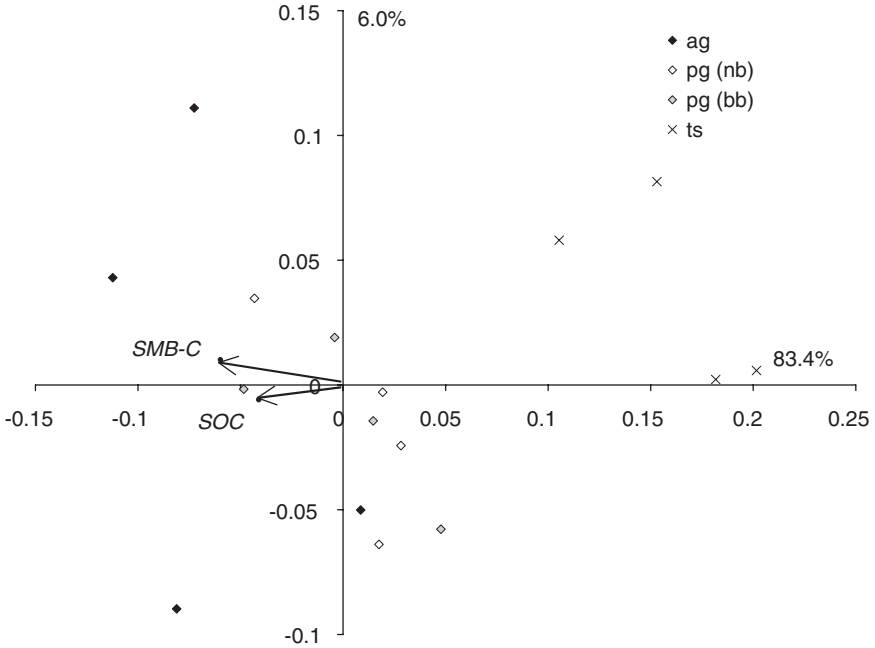

Fig. 3. CCA ordination of PLFAs extracted from soil in the 0–15 cm layer in annual grassland (ag), in restored perennial grassland (pg), and tilled bare soil (ts) at Hastings Reserve in Carmel Valley, California. Perennial grassland was sampled in the 'near bunchgrass' (nb) and in 'between bunchgrass' (bb) zones. Plant cover in the 'between bunchgrass' zone consisted of annual grasses ($n = 4$). Soil microbial biomass-C (SMB-C) and soil organic carbon (SOC) are indicated by vectors.

bunchgrass' and 'between bunchgrass' zones of the restored perennial grassland, concentrations of specific PLFA were significantly reduced in the tilled bare plot. This same trend was observed among summations of PLFA biomarkers for specific functional groups (e.g., actinomycetes, Gram$^+$, Gram$^-$, and fungi; Table 1).

When viewed as a percentage of total PLFA, certain groups of microbes differed across treatments, based on putative identities of specific PLFA (Table 1). In all treatments, the relative proportion of biomarkers corresponding to actinomycetes increased with depth, reaching a maximum value (i.e., 17–19% of total PLFA) in the

60–80 cm depth. For the upper layer (0–15 cm), the 'between bunchgrass' zone of the restored perennial grassland, had a lower proportion of actinomycetes than in the tilled bare soil (i.e., 9.6% vs. 11.1% of total PLFA, respectively; $P = 0.05$).

Gram$^-$ biomarkers (nmol g^{-1}) showed significant treatment differences in the 15–30 cm layer only (Table 1). The proportion of Gram$^-$ biomarkers in the tilled bare soil plot (approx. 11%) was slightly but significantly lower than in annual and perennial grassland soils, where Gram$^-$ biomarkers ranged from up to 14% in the two upper layers to approximately 8% in the 60–80 cm layer.

Gram$^+$ biomarkers (excluding actinomycetes) represented the largest proportion of bacterial PLFAs. In the surface layer of annual grassland, Gram$^+$ biomarkers were significantly lower than in perennial grassland plots or the tilled bare soil plot (20% vs. 22–23%, respectively). With increasing depth, the relative proportion of Gram$^+$ PLFAs increased slightly in all treatments. A significant increase was only observed in the annual grassland (20% at 0–15 cm vs. 25% at 60–80 cm) and in the 'near bunchgrass' zone of the restored perennial grassland (22% at 0–15 cm vs. 31% at 60–80 cm).

Fungal PLFA biomarkers showed strong and significant decreases with increasing depth in the untreated annual grassland and the restored perennial grassland. However, the surface layer of the tilled bare soil treatment supported significantly lower proportions of fungal PLFAs (24%) than in annual or restored perennial grasslands (31% and 28–30%, respectively), and fungal biomarkers decreased to 15% in the lowest layer (60–80 cm) of the tilled bare soil treatment. PLFAs of unknown affiliation ranged between 24% of the total in the surface layer of grassland soils and 30% in samples from the 60–0 cm layer of the tilled bare plots (Table 1).

Measures of ergosterol, a non-PLFA fungal biomarker, were largely consistent with fungal PLFAs (mol g^{-1}) (Table 2). In the surface layer, ergosterol was significantly lower in tilled bare soils than in grassland soils. In all treatments, ergosterol content decreased with increasing depth as found for fungal PLFAs. However, in the 15–30 cm layer, ergosterol content was found to be significantly higher in the annual grassland compared to the other treatments.

All four PLFAs indicative of fungal biomass were in good correspondence with the ergosterol contents as drawn from several correlations (data not shown). Fungal marker 18:3ω6c was strongly related to the presence of plants, but was limited to the uppermost layer. Its frequency in the grassland soils was 22%, while the tilled bare plot did not contain the fungal marker 18:3ω6c.

Table 2
Ergosterol concentrations as an additional marker for fungi biomass for 0–80 cm soil profiles in annual grassland (ag), in restored perennial grassland (pg), and tilled bare soil (ts). Perennial grassland was sampled in 'near bunchgrass' (nb) zones and in 'between bunchgrass' (bb) zones ($n = 4$, standard deviation (SD) and statistical grouping (sg) by Tukey HSD test; different letters indicate different groupings)

Depth (cm)		Fungi (ergosterol, μg g^{-1})		
		Mean	SD	sg
0–15	ag	0.48	0.12	b
	pg (bb)	0.50	0.11	b
	pg (nb)	0.47	0.08	b
	ts	0.30	0.04	a
15–30	ag	0.27	0.08	b
	pg (bb)	0.10	0.05	a
	pg (nb)	0.09	0.01	a
	ts	0.12	0.02	a
30–60	ag	0.06	0.05	
	pg (bb)	0.03	0.04	
	pg (nb)	0.02	0.01	
	ts	0.03	0.01	
60–80	ag	0.03	0.02	
	pg (bb)	0.03	0.02	
	pg (nb)	0.01	0.00	
	ts	0.01	0.01	

4. Discussion

4.1. Microbial community composition and grassland restoration

Grassland restoration that utilizes agricultural practices can change ecosystem processes to support the survival of native plants. Agricultural practices, such as tillage, weeding and the use of herbicide reported here, have been clearly linked to changes in the composition and performance of microbial communities (Ka et al., 1995; Calderón et al., 2001). In this study, PLFA profiles in the restored perennial grassland became more similar to those in the old field annual grassland than the tilled bare soil, as did SMB-C and microbial activity (cf. Potthoff et al., 2005a). This increasing similarity suggests microbial resilience to the disturbance caused by 2 years of tillage prior to seeding perennials. This was particularly evident for fungal markers, but all functional groups were more abundant in the surface soil of grasslands compared to continuously tilled bare soil.

The presence of vegetation appears to have facilitated the recovery of the soil environment and the microbial community compared to tilled bare soil, but the plant species composition of the grasslands was less important. Other recent evidence suggests that soil microbial communities may change in response to invasion by exotic plant species, such as annual grasses (Kourtev et al., 2002, 2003; Callaway et al., 2004). Abiotic and biotic shifts in environmental conditions can occur during plant invasions, including changes in water availability, temperature, phenology, and plant tissue quality and root carbon deposition (Levine et al., 2003; Hütsch et al., 2002). Depending on the magnitude of these environmental and biological changes, microbial communities might show either slight or strong shifts in composition in response to a change in plant species. After perennial grassland restoration, only minor changes occurred in cover and plant

biomass (Potthoff et al., 2005a; Steenwerth et al., 2005). Thus, the minor response of microbes to the two grassland treatments may be attributed partially to the lack of profound effects of plant species on the soil microenvironment, at least during the early stages after restoration was completed.

The finding that the microbial communities did not segregate by grassland type seems to contradict our earlier study conducted at the landscape scale in the Central Coast region, in which microbial communities of annual grasslands differed from perennial grasslands, and restored perennial grasslands differed from native, undisturbed perennial grasslands (Steenwerth et al., 2003). However, young perennial bunchgrasses may impose less impact on soil microbial communities at this restored perennial grassland due to their smaller size, compared to those in nearby long-term restored and undisturbed perennial grasslands in the previous study. Furthermore, bunchgrasses in the restored perennial grassland were surrounded by annuals, so that even the 'near bunchgrass' samples contained a high density of annuals (Potthoff et al., 2005a; Steenwerth and Jackson, personal observation). A stronger effect of perennial bunchgrasses in the restored grassland on soil microbial communities is expected with time because their biomass will increase. As observed in relict stands of native perennial bunchgrasses, bunchgrasses appear to reduce other grasses and forbs, forming a 'checkerboard pattern' of perennial grasses and interstitial zones with low cover of annual species (Hamilton et al., 2002; Corbin and D'Antonio, 2004). In addition, perennial bunchgrasses will deposit litter of different quality, e.g., higher C/N ratios compared to annual grasses, which may alter decomposition processes and thus influence the soil microbial community composition (Hooper and Vitousek, 1998; Hooper et al., 2000; Mack and D'Antonio, 2003; J. Corbin, personal communication). Unfortunately, no relict perennial grasslands existed adjacent to this restoration plot for comparison.

Fungi showed the most pronounced change associated with management practices. Fungal populations decrease in response to physical disturbances like tillage (Guggenberger et al., 1999), are known to be the first colonizers on grass litter with wide C/N ratios (Bowen and Harper, 1990), and are sensitive to dry conditions (Frey et al., 1999). Strong associations between ergosterol, a general biomarker of fungal biomass (Djajakirana et al., 1996) and PLFA fungal markers underscore the effect of plant presence on shifting the soil microbial community in the surface soils. Despite potential differences in plant tissue quality, root deposition and phenology between perennial bunchgrasses and annual grasses, little differentiation in fungal associations occurred between the annual and perennial grassland treatments. In a previous study investigating soil microbial community composition along a gradient in land use intensity in this region (Steenwerth et al., 2003), the same fungal markers were enriched in grassland soils except for 18:3ω6,9,12c, which displayed strong associations with hayfields and intensively cultivated fields supporting cole crops. In the current study, 18:3ω6c, which is not found in plant tissue (Zelles, 1997), was only present in grassland soils. This occurred especially in the surface where root length and biomass were greatest, indicating that fungi enriched with this marker may be largely involved with decomposition of plant litter. This confirms the important role of fungi as part of the zymogenic microbial biomass at the litter–soil interface (Frey et al., 2003; Potthoff et al., 2005b), and to handle low C/N ratios in plant residues (Paul and Clark, 1996; Paustian and Schnürer, 1987).

4.2. *Microbial community composition as a function of soil depth*

While one might expect changes in microbial communities along the vertical soil profile due to soil disturbance and with shifts in plant community composition because perennial bunchgrasses support higher root biomass at greater depth than annual grassland species, this trend was not observed. In fact, only slight differences in the distribution of root length and root biomass were observed for these young perennial plants (Potthoff et al., 2005a). Reductions in soil C content, which occur when soil is tilled as it was during grassland restoration, are associated with lower soil aggregation and poorer soil structure, causing a less favorable environment for root growth (Kay, 1990).

Unlike many studies investigating indices of soil microbial activity and composition, the current investigation includes both the upper and lower soil layers. Reductions in both the availability of oxygen and the quality and availability of soil organic C, and less extreme changes in temperature and moisture are correlated with increasing depth (Lavahun et al., 1996; Ehleringer et al., 2000). Soil moisture regime, temperature and soil C availability are prominent factors in determining soil microbial community composition (Zogg et al., 1997; Fierer et al., 2003; Drenovsky et al., 2004; Steenwerth et al., 2005). Extreme temperature changes and repeated wetting and drying, a cycle that is common in surface soils in Mediterranean climates, can both influence soil microbial community composition and likely are two factors influencing the selection of distinct soil microbial communities in the surface compared to lower layers (Lundquist et al., 1999; Schimel et al., 1999).

Soil C availability has been proposed as a driving force behind the distribution of soil microorganisms along the vertical soil profile (Fierer et al., 2003). Although measures of soil C availability, labile and recalcitrant C pools (i.e., root biomass and length, total C, SMB-C, and soil respiration; Potthoff et al., 2005a), and total PLFA declined with increasing depth in all treatments, they did not explain the distribution of microbial communities according to depth (Fig. 2). Instead, a soil moisture gradient was associated with differences in soil microbial communities in the surface and lower depths.

Shifts in the relative proportions of specific biomarkers are consistent with previous studies investigating alterations in microbial communities along the vertical soil profile. Actinomycetes have the ability to mineralize very recalcitrant organic compounds under conditions of low oxygen concentrations or anaerobic conditions (Goodfellow and Williams, 1983), which may explain their relative increase in subsurface depths where soil C recalcitrance increases (Trumbore, 2000). Gram$^+$ bacteria including actinomycetes can be classified as part of the autochthonous soil microbial community associated with lower availability of organic C substrates (Griffiths et al., 1999; Fierer et al., 2003) rather than the zymogenous organisms (i.e., Gram$^-$ bacteria and fungi) that strongly depend on the input of fresh organic material to create hot spots of decomposition in soil (Griffiths et al., 1999; Potthoff et al., 2005b). In all treatments, the proportion of actinomycetes and Gram$^+$ bacteria, as expected, increased with depth while the proportion of fungi and Gram$^-$ bacteria correspondingly decreased. Quite similar effects of soil depth as a function controlling microbial community composition were also found by Fierer et al. (2003) for California grassland and by Feng et al. (2003) for continuous cotton plantings.

While this study demonstrated that soil microbial communities displayed resilience to the disturbance caused during restoration of annual grasslands to native perennial grasslands, and that the distribution of soil microbial groups along the vertical soil profile was relatively consistent with previous studies, it remains to be seen whether soil microbial community composition in the restored grassland will further distinguish itself from the annual grassland on a long-term timescale to approach that of relict perennial grasslands. Here, the findings indicate that soil microbial communities of the restored grassland closely resemble their annual grassland counterparts despite the presence of the native perennial bunchgrass, at least in first few years after the completion of restoration.

5. Conclusion

We conclude that restoration measures that use agricultural methods had strong but temporary effects on microbial communities in the soil limited to the uppermost layer. Our results indicate a high resilience of microbial communities after restoration as microbial community composition in a restored perennial grassland was more similar to that in annual grassland than tilled, bare soil. Soil depth provided a strong gradient of changing habitat conditions as reflected by changes in PLFA profiles. In addition, microbial communities of deeper layers were not affected by surface management inputs and disturbance. In this study, 4 years after planting native perennial bunchgrasses, the only specific effect of native grasses on microbial communities were changes in PLFA profiles of the surface layer.

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