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Soil microbial community composition and land use history in cultivated and grassland ecosystems of coastal California

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Abstract

Phospholipid ester-linked fatty acid (PLFA) profiles were used to evaluate soil microbial community composition for 9 land use types in two coastal valleys in California. These included irrigated and non-irrigated agricultural sites, non-native annual grasslands and relict, never-tilled or old field perennial grasslands. All 42 sites were on loams or sandy loams of similar soil taxa derived from granitic and alluvial material. We hypothesized that land use history and its associated management inputs and practices may produce a unique soil environment, for which microbes with specific environmental requirements may be selected and supported. We investigated the relationship between soil physical and chemical characteristics, management factors, and vegetation type with microbial community composition. Higher values of total soil C, N, and microbial biomass (total PLFA) and lower values of soil pH occurred in the grassland than cultivated soils. The correspondence analysis (CA) of the PLFA profiles and the canonical correspondence analysis (CCA) of PLFA profiles, soil characteristics, and site and management factors showed distinct groupings for land use types. A given land use type could thus be identified by soil microbial community composition as well as similar soil characteristics and management factors. Differences in soil microbial community composition were highly associated with total PLFA, a measure of soil microbial biomass, suggesting that labile soil organic matter affects microbial composition. Management inputs, such as fertilizer, herbicide, and irrigation, also were associated with the distinctive microbial community composition of the different cultivated land use types.

Keywords: Microbial communities; Land use history; Grasslands; Cultivation; PLFA

1. Introduction

Understanding soil biology and ecology is increasingly recognized as important for the restoration and sustainability of ecosystems. In all ecosystems, soil microbes play important roles in decomposition of organic matter, nutrient cycling, and plant nutrient availability (Paul and Clark, 1989). The activity and biomass of microbes respond to soil management, organic matter, and the abiotic environment, and are influenced by plant litter and rhizosphere effects (Zaady et al., 1996; Hooper and Vitousek, 1998; Jones, 1998; Calderón et al., 2000; Chen and Stark, 2000). Recent landscape-level studies have shown relationships between soil microbial community composition, and biotic and abiotic environmental conditions (Waldrop et al., 2000; Yin et al., 2000; Myers et al., 2001). A gradient of increasing intensity of disturbance represented by a range of land use histories can capture the variation across the landscape, within a landscape unit, and in physical and chemical characteristics of soil, management, and vegetation, and may provide insights into the complex set of factors that affect soil microbial biomass and community composition.

Grasslands and cultivated soils represent two types of land use that have distinct effects on soil characteristics and soil microbiology. A strong decline in soil carbon (C) occurs after repeated tillage (Schimel et al., 1985; Elliott, 1986; Burke et al., 1989; Woods, 1989; Conant et al., 2001), while grasslands tend to support increased soil C and microbial biomass with greater spatial heterogeneity within the soil profile than in cultivated soils (Woods, 1989; Kandeler and

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Murer, 1993; Calderón et al., 2000). Agricultural practices such as residue incorporation, cropping sequence, irrigation, and tillage alter soil microbial biomass (Anderson and Gray, 1990; Sparling et al., 1994; Franzluebbers et al., 1995) and soil microbial community composition (Bossio et al., 1998; Lundquist et al., 1999; Calderón et al., 2000). In some cases, cultivation history has long-term effects on microbial community structure in abandoned agricultural fields (Buckley and Schmidt, 2001), and gradients in soil fertility in either grasslands (Donnison et al., 2000; Grayston et al., 2001) or cultivated sites (Yao et al., 2000) have been shown to influence microbial community composition. However, less is known about concomitant changes in soil microbial community composition due to multiple factors (i.e. physical and chemical soil characteristics, vegetation and management) associated with differences between grasslands and agriculture at the landscape scale.

Phospholipid ester-linked fatty acid (PLFA) analysis uses the cell membrane lipids within microorganisms as biomarkers for specific groups of organisms, thereby creating a profile or fingerprint of the microbial community. Certain fatty acids are unique markers for specific microbial groups or are indicators of microbial stress (Guckert et al., 1986). The total concentration of PLFA is a measure of viable microbial biomass, since phospholipids are readily degraded after cells die (Zelles, 1997). PLFA is a more quantitative measure of microbial communities than most current DNA fingerprinting methods (Muyzer et al., 1993; Kowalchuk et al., 1997). Direct counts and BiOLOG (Zak et al., 1994; Buyer and Drinkwater, 1997), also used to describe microbial communities, provide limited information since their culture environment differs from the soil environment, and selected dilutions of the microbial inoculum may influence results in BiOLOG (Bossio and Scow, 1998; Smalla et al., 1998).

Diverse grassland and cultivated ecosystems in the central coast region of California provide a gradient of increasing intensity of soil disturbance in which to compare the effects of land use on microbial community structure. Land use history and its associated management inputs and practices may create a unique soil environment, for which microbes with specific environmental requirements may be selected and supported. Different land use histories would thus be associated with characteristic microbial communities, although a range of microbial community composition may exist within a single land use type. Specifically, we will examine the effects of (1) soil chemical and physical characteristics, (2) management factors, and (3) vegetation type (grassland or cultivated) that are associated with specific land use histories on microbial community composition.

A landscape approach is used to survey various grassland and cultivated ecosystems with different land use histories for soil characteristics and microbial community composition as described by multivariate analysis of PLFA. A few relict stands of perennial bunchgrasses still exist in the central coast region of California (Stromberg and Griffin, 1996). Most grasslands, however, are dominated by annual plants from the Mediterranean Basin, which colonized after overgrazing in the 1800s (Beetle, 1947; Burcham, 1957). The region also supports grain production systems without irrigation, many of which were abandoned to become annual grassland when grain production became less profitable. Much of the land is committed to high input, intensive production of vegetables. In landscape-level studies, the variation in soil texture and associated soil characteristics can augment the difficulty of identifying specific factors that influence soil microbial community composition within or between landscape units. To achieve high resolution and decrease effects of soil type along a gradient of soil disturbance associated with different land use histories, the ecosystem types were selected from similar sandy loam soils derived from granite and alluvial parent material. Our objectives are to (1) determine whether different land use histories on similar soil types lead to differences in microbial community composition, and (2) discern distinct relationships between soil characteristics, management factors, vegetation type and soil microbial community composition.

2. Materials and methods

2.1. Site description and selection

In the Salinas and Carmel Valleys of Monterey County, CA, the Mediterranean climate has mean summer and winter temperatures, respectively, of 15.5 and 13 °C (Cook, 1978). Mean annual precipitation is between approximately 350 and 500 mm. Locations of sites ranged from the Salinas Valley at 20 m elevation to the Upper Carmel Valley at 556 m elevation. The sites were located along a transect of approximately 40 km, moving eastward from the town of Carmel Valley to the hills east of Gonzales (Fig. 1). The 42 sites sampled in this study were on alluvial soils derived largely from granite parent material. Soil types include the Chualar (Fine–loamy, mixed, thermic Fluventic Haploxeroll), Gorgonio (Sandy, mixed, thermic Fluventic Haploxeroll), and Sheridan (Coarse–loamy, mixed, thermic Pachic Haploxeroll) sandy loams. These soil series are classified on the basis of the whole pedon, and although they have different subsoil characteristics, their surface horizons are morphologically similar.

In choosing sites, we aimed for consistency in soil texture (sandy loams) and slope (3–4%). Each site was classified into land use types indicative of current land utilization. Four main types of systems were recognized: irrigated and non-irrigated agricultural systems, and annual and perennial grasslands. These four groups were further subdivided into the following 9 land use types (Table 1), described here in order of high to low intensity of disturbance.

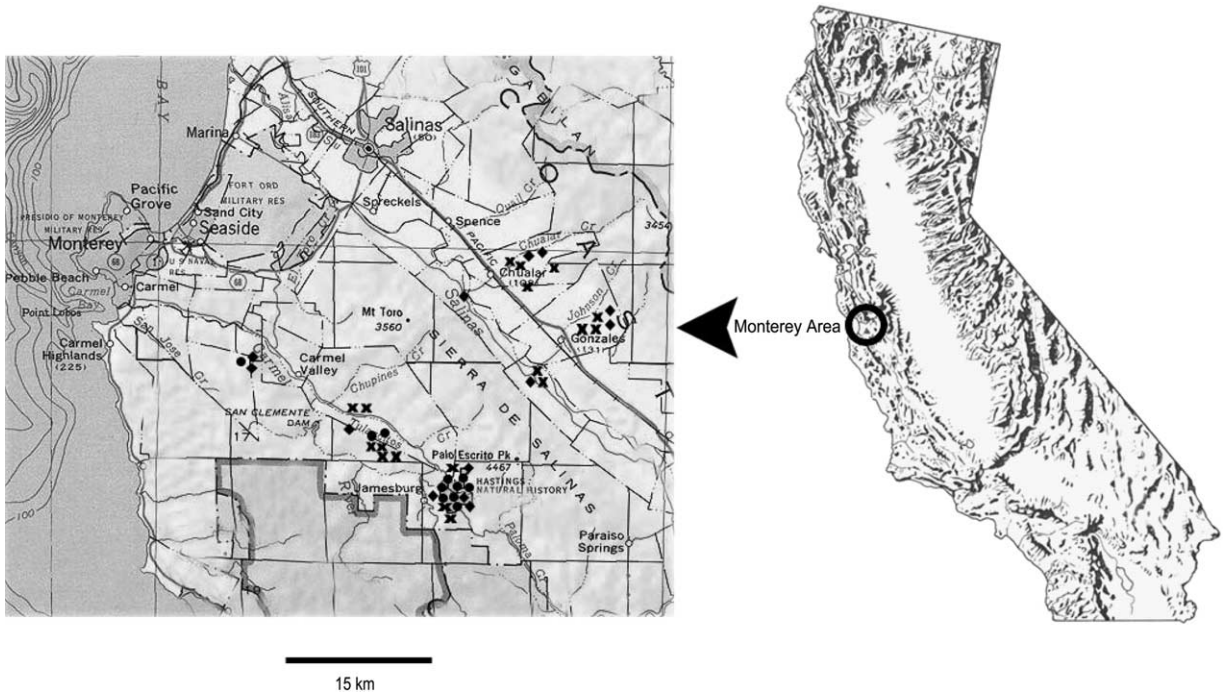

Fig. 1. Sites in the Carmel and Salinas Valleys in Monterey County, CA that were surveyed for their respective land use histories. Symbols indicate land use types as follows: (●) Perennial grasslands, i.e. PerGrass, PerGrass Oldfield and PerGrass + GRAZ; (◆) cultivated sites, i.e. Vegetable, Hayfield, Fallow, PerGrass Ag; (✖) Annual grasslands, i.e. AnnGrass + GRAZ and AnnGrass.

Irrigated agriculture includes intensively cultivated vegetable fields ('Vegetable') and semi-permanent native perennial bunchgrass (*Koelaria macrantha* and *Nassella pulchra*) stands grown for seed production under less intensive cultivation practices ('PerGrass Ag'). All irrigated sites received fertilizer and herbicide applications, and pesticides were also applied to the Vegetable sites. Non-irrigated agriculture consists of hay fields ('Hayfield') and three cultivated sites that were fallow ('Fallow'). The Hayfield sites supported crops such as barley and oats, and received fertilizer and herbicide. The Fallow sites were formerly annual grasslands that had been tilled and received herbicide for two consecutive years, and plant cover was absent for this period. All cultivated sites experienced tillage in the year prior to sampling. In the PerGrass Ag sites, which were cultivated to produce native grass seed for restoration projects, only the bed shoulders were frequently tilled, and perennial bunchgrasses had been planted 3 years prior to sampling. Plant cover in the cultivated sites was often quite low, depending on whether the site was sampled when the crop was present.

Non-native annual grasses from the Mediterranean Basin dominated annual grassland sites. All annual grassland sites had been tilled at some point in the last 100 years. Ungrazed annual grassland ('AnnGrass') refers to sites without livestock grazing but with native herbivores. Other sites were also grazed by cattle ('AnnGrass + GRAZ'). These sites were last cultivated between 8 and 70 years ago, and were abandoned to become annual grassland. In contrast, perennial grass old fields ('PerGrass Oldfield') were formerly cultivated, last tilled from 6 to 43 years ago, and now support planted stands of either native or non-native perennial bunchgrasses. Relict, never-tilled stands of native perennial bunchgrasses either did ('PerGrass + GRAZ') or did not support cattle grazing ('PerGrass'). No grassland sites received irrigation, fertilizer, herbicide or pesticide.

Information on land use history and current management practices was obtained by interviewing farmers and ranchers. Data included the time since last tillage, presence of livestock grazing, and application of herbicide, pesticide and irrigation, but did not give details regarding the frequency or amounts used during these management practices. Oral histories from landowners, historical records, and old aerial photos were gathered to determine the cultivation and management histories on the grassland stands in Carmel Valley (Stromberg and Griffin, 1996).

2.2. Soil sampling and analysis

The survey of 42 sites was conducted during February and March of 1998. Sites were assigned one of 17 possible dominant plant species categories. Because some sites had multiple dominants, and not all plants were identifiable to the species level, our categories referred to either plant species or to plant community composition (e.g. *N. pulchra*, *Elymus glaucus*, annual grass + forbs, *Hordeum vulgare*, *Brassica oleracea*, grass/legume cover crop, and bare soil). Four cores were taken per site from an area of approximately

Table 1
Soil characteristics of the 42 agricultural and grassland sites in the Salinas and Carmel Valleys of Coastal California. Means and SE are shown for the 0–6 and 6–12 cm depths of each of the land use types. ND = No data

Land use type[a]		Sand ($g\ g^{-1}$)	Silt ($g\ g^{-1}$)	Clay ($g\ g^{-1}$)	Bulk density ($g\ cm^{-3}$)	pH	X-K (meq $100\ cm^{-3}$)	X-Ca (meq $100\ cm^{-3}$)	X-Mg (meq $100\ cm^{-3}$)	H_20 ($g\ g^{-1}$)	Total soil C ($mg\ cm^{-3}$)	Total soil N ($mg\ cm^{-3}$)	Total PLFA ($\mu g\ g^{-1}$)
Irrigated agriculture (0–6 cm)													
Vegetable ($n = 5$)	X	0.65	0.24	0.11	1.49	7.5	0.8	29.1	3.3	0.128	17.48	1.75	10.8
	SE	0.05	0.03	0.02	0.09	0.1	0.2	6.2	0.6	0.016	4.19	0.29	2.7
PerGrass Ag ($n = 2$)	X	0.71	0.22	0.07	1.28	6.3	0.6	7.3	2.3	0.109	11.25	1.06	10.1
	SE	0.09	0.06	0.03	0.13	0.2	0.1	2.0	0.7	0.014	1.18	0.13	2.8
Non-irrigated agriculture (0–6 cm)													
Fallow ($n = 3$)	X	0.84	0.11	0.05	1.32	5.8	0.3	5.9	1.7	0.070	12.32	1.08	7.4
	SE	0.02	0.02	0.01	0.18	0.0	0.1	0.7	0.8	0.007	0.66	0.10	0.2
Hayfield ($n = 6$)	X	0.67	0.25	0.08	1.46	6.3	0.6	7.0	2.0	0.131	15.44	1.63	16.4
	SE	0.05	0.05	0.01	0.10	0.3	0.1	1.1	0.5	0.013	1.48	0.11	1.9
Annual grasslands (0–6 cm)													
AnnGrass + GRAZ ($n = 10$)	X	0.65	0.27	0.08	1.39	5.7	0.9	7.9	1.8	0.282	30.58	3.04	32.0
	SE	0.3	0.02	0.01	0.06	0.1	0.1	1.3	0.4	0.033	2.80	0.27	2.3
AnnGrass ($n = 6$)	X	0.77	0.16	0.06	1.20	6.1	0.4	7.2	1.5	0.136	15.42	1.38	22.0
	SE	0.04	0.03	0.01	0.06	0.1	0.1	0.8	0.2	0.013	0.84	0.08	1.9
Perennial grasslands (0–6 cm)													
PerGrass Oldfield ($n = 6$)	X	0.76	0.16	0.07	1.25	6.0	0.6	7.2	1.3	0.179	25.83	2.50	45.6
	SE	0.04	0.03	0.01	0.05	0.1	0.1	1.3	0.5	0.024	3.44	0.28	7.8
PerGrass + GRAZ ($n = 2$)	X	0.68	0.24	0.08	1.00	5.5	0.5	4.8	1.3	0.273	27.0	2.00	34.7
	SE	0.08	0.02	0.01	0.10	0.2	0.0	1.1	0.1	0.014	3.0	0.0	4.3
PerGrass ($n = 2$)	X	0.47	0.35	0.19	0.96	5.3	0.7	8.9	2.1	0.287	18.85	1.89	31.1
	SE	0.13	0.10	0.03	0.07	0.5	0.1	3.4	0.2	0.068	2.13	0.20	3.7
Irrigated agriculture (6–12 cm)													
Vegetable ($n = 5$)	X	0.70	0.22	0.08	1.55	7.3	0.9	25.0	3.4	ND	19.01	1.85	ND
	SE	0.03	0.03	0.03	0.09	0.3	0.2	5.7	0.7	ND	4.84	0.34	ND
PerGrass Ag ($n = 2$)	X	0.75	0.19	0.07	1.20	6.1	0.5	5.7	1.9	ND	11.61	1.01	ND
	SE	0.09	0.07	0.03	0.03	0.2	0.1	1.8	0.6	ND	2.56	0.19	ND
Non-irrigated agriculture (6–12 cm)													
Fallow ($n = 3$)	X	0.85	0.11	0.04	1.32	5.8	0.3	6.6	1.9	ND	12.03	1.05	ND
	SE	0.04	0.01	0.01	0.18	0.0	ND	ND	ND	ND	0.50	0.01	ND
Hayfield ($n = 6$)	X	0.67	0.25	0.08	1.50	6.4	0.6	7.6	2.1	ND	14.70	1.59	ND
	SE	0.05	0.04	0.01	0.01	0.4	0.1	1.4	0.5	ND	1.08	0.10	ND
Annual grasslands (6–12 cm)													
AnnGrass + GRAZ ($n = 10$)	X	0.65	0.27	0.08	1.51	5.6	0.8	6.8	1.6	ND	24.96	2.48	ND
	SE	0.03	0.02	0.01	0.05	0.2	0.1	1.3	0.4	ND	3.40	0.30	ND
AnnGrass ($n = 6$)	X	0.76	0.17	0.07	1.29	6.1	0.5	8.8	1.9	ND	14.26	1.32	ND
	SE	0.14	0.03	0.02	0.07	0.1	0.1	1.5	0.4	ND	0.97	0.10	ND

Table 1 (*continued*)

Land use type[a]		Sand ($g\ g^{-1}$)	Silt ($g\ g^{-1}$)	Clay ($g\ g^{-1}$)	Bulk density ($g\ cm^{-3}$)	pH	X-K (meq $100\ cm^{-3}$)	X-Ca (meq $100\ cm^{-3}$)	X-Mg (meq $100\ cm^{-3}$)	H_20 ($g\ g^{-1}$)	Total soil C ($mg\ cm^{-3}$)	Total soil N ($mg\ cm^{-3}$)	Total PLFA ($\mu g\ g^{-1}$)
Perennial grasslands (6–12 cm)													
PerGrass Oldfield ($n = 6$)	X	0.75	0.17	0.08	1.36	6.0	0.7	6.6	1.5	ND	16.53	1.79	ND
	SE	0.04	0.03	0.01	0.03	0.1	0.1	1.7	0.6	ND	2.51	0.18	ND
PerGrass + GRAZ ($n = 2$)	X	0.68	0.22	0.10	1.30	5.3	0.6	5.3	1.6	ND	22.32	2.19	ND
	SE	0.10	0.02	0.00	0.01	0.2	0.1	0.2	0.2	ND	4.12	0.68	ND
PerGrass ($n = 2$)	X	0.44	0.35	0.22	1.18	5.2	0.8	7.9	3.5	ND	16.28	1.80	ND
	SE	0.14	0.12	0.03	0.10	0.5	0.0	0.7	0.9	ND	0.51	0.08	ND

[a] Vegetable = Intensive cultivation of crops; PerGrassAg = Low intensity cultivation of crops; Fallow = Tilled annual grassland without plants; Hayfield = Hayfields; AnnGrass + GRAZ = Grazed annual grassland; AnnGrass = Ungrazed annual grassland; PerGrass Oldfield = Perennial grassland old field; PerGrass + GRAZ = Grazed perennial grassland; PerGrass = Ungrazed relief perennial grassland.

20 m^2 between 3 and 7 days after rainfall to achieve similar moisture availability. When plants were present, cores were placed over the crown of plants of the dominant species, so that sampled soil contained roots of these species. Cores were obtained from the 0–12 cm depth by gently pounding brass rings (8.5 cm diameter × 6 cm depth) into the ground. Soils were then air-dried, large roots (>1 mm) were removed with tweezers, and soils were sieved through 2 mm mesh screens. Rocks >2 mm were weighed. Sieved soils from each site were analyzed for pH by saturated paste (US Salinity Laboratory, 1954), and particle size distribution by the method of Gee and Bauder (1986). The exchangeable calcium (X-Ca) and magnesium (X-Mg) were analyzed by inductively coupled plasmic atomic emission spectrometry (Soltanpour et al., 1982). Exchangeable potassium (X-K) was measured by emission spectroscopy (Knudsen et al., 1982). Total soil C and N were determined by the combustion gas analyzer method (Pella, 1990). Bulk density was calculated from the dry mass of soil per volume collected in the brass ring. All analyses, except bulk density and moisture content, were performed by the Department of Agriculture and Natural Resources Analytical Laboratory at the University of California at Davis. Moisture retention curves were developed with a pressure plate apparatus for soil taken from one site of each of the following land use types: AnnGrass + GRAZ, PerGrass + GRAZ, Vegetable, and PerGrass Ag. To estimate mean soil matric potential for each of the 42 sites, gravimetric moisture was interpolated from the moisture retention curve of the soil from the most closely corresponding land use type.

A separate set of samples was simultaneously obtained for PLFA analysis. Four small cores of 13.5 cm^3 (1.5 cm × 1.5 cm × 6 cm deep) per site were taken from the same points as the larger samples. These cores were removed with as little disturbance and mixing as possible, combined into a single sample, and immediately placed on ice for transport. This soil was stored in the laboratory at −20 °C until extraction.

2.3. PLFA analysis

Immediately before PLFA analysis, the soil from each frozen sample was mixed, and all visible root fragments were removed with tweezers. Three subsamples were analyzed per field sample, i.e. per site. A sample was also taken for gravimetric moisture by drying soil at 105 °C for 48 h. A complete description of the procedure for PLFA extraction and analysis is detailed in Bossio and Scow (1995). Total lipids were extracted from moist soil (equivalent to 8.0 g of dry soil) by a chloroform–methanol extraction (Bligh and Dyer, 1959) modified to incorporate a 0.05 M phosphate buffer. The PLFA were then purified from the lipid 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). The peaks were identified using

the bacterial standards and identification software from the microbial identification system (Microbial ID, Inc., Newark, DE). The samples were analyzed using the Microbial ID protocol, modified to include an internal standard (19:0) of known concentration. The fatty acids were quantified by comparison of the peak areas with those of the standard peak. Fatty acid terminology utilizes 'A:B ωC' where 'A' indicates the total number of carbon atoms, 'B' the number of unsaturations, and 'ω' precedes 'C', the number of carbon atoms between the closest unsaturation and the aliphatic end of the molecule. The suffixes *c* and *t* indicate *cis* and *trans* geometric isomers. The prefixes 'i' and 'a' refer to iso and anti-iso methyl branching. Hydroxy groups are indicated by 'OH'. Cyclopropyl groups are denoted by 'cy'. 10 Me refers to a methyl group on the tenth carbon from the carboxylic end of the fatty acid. The total extractable PLFA provides a measure of microbial biomass at each site (Zelles et al., 1995).

2.4. Statistical analysis

Comparison of means of soil characteristics for different land use types (*t*-tests), and linear regressions between total PLFA and soil characteristics were performed in SAS (SAS Institute Inc., 1991). Tests were considered significant at $P \leq 0.05$.

Multivariate techniques were used to analyze the data for soil characteristics, management inputs, and PLFA data (as ng PLFA g^{-1} soil) using CANOCO, version 4.0 (Microcomputer Power, Inc., Ithaca, NY). In graphical outputs, the position of the sites along the axes is determined by the loading scores, which describe the relative importance of a variable along the ordination axis. Land use types within a cluster on a graph are more similar to each other in terms of their sampled variables than other sites outside of that cluster. Only the 32 PLFA that were consistently present at all sites were included in the multivariate analyses. However the total PLFA detected in each sample ranged between 32 and 80 distinct molecules.

CA was used to test the ability of PLFA data to distinguish between each of the 9 land use types. CA is appropriate for use with data sets containing nominal and zero values, which occur in many ecological studies, and attempts to extract the underlying unimodal environmental gradient in the data set. The CA algorithm constructs a theoretical variable that best explains the data (i.e. PLFA) for each ordination axis, and uses reciprocal averaging to assign values to the sites to maximize the dispersion of the scores for the PLFA or management variables. Further ordination axes, which are uncorrelated with the previous axis, are constructed to explain the remaining variation.

The relationship between microbial community composition, soil characteristics and management inputs was analyzed by canonical correspondence analysis (CCA). CCA permits direct analysis of PLFA profiles in relation to specific environmental variables (e.g. soil characteristics, site and management factors). It constrains ordination axes to be linear combinations of environmental variables, and will maximize the dispersion of the PLFA scores (ter Braak, 1987). The management inputs are represented by a centroid. Its position is indicative of the relationship between a specific management input and either of the ordination axes. Soil characteristics are represented by vectors. Vectors of greater magnitude and forming smaller angles with an ordination axis are more strongly correlated with that ordination axis. High scores of absolute value for a given PLFA or a given site on a CCA axis indicate that it is highly related to the axis and to the environmental variable exhibiting high correlation to the axis. All soil characteristics and management variables were tested for significant contribution to the explanation of the variation in the PLFA data with the Monte Carlo permutation test associated with the forward selection subroutine in CANOCO. Only variables that were significant by the Monte Carlo permutation test at the $P \leq 0.05$ level are included in the CCA biplot.

3. Results

3.1. Soil characteristics and microbial biomass

Soil texture was generally similar for the 9 land use types (Table 1). All soils were sandy loams with $<15\%$ clay content, except for three sites (one each for PerGrass, Hayfield and Vegetable) that were loams. The means of the particle size distribution of the top 0–6 cm depth of the four major groups of sites (i.e. irrigated vs. non-irrigated agriculture, and annual vs. perennial grasslands) were not statistically different (*t*-tests, $P \leq 0.05$, data not shown). Particle size distribution and most of the other soil characteristics (see later) were very similar between the 0–6 and 6–12 cm depths.

Bulk density means generally ranged from 1.2 to 1.5 g cm^{-3}, with little distinction between cultivated, grassland, or grazed sites (Table 1). The exceptions were the PerGrass + GRAZ and PerGrass types, in which the root zone of *N. pulchra* plants was sampled; mean bulk density of these soils was approximately 1 g cm^{-3}. Bulk density tended to be higher in grazed rather than ungrazed annual grasslands, and in perennial grass old fields compared to never-tilled perennial grasslands.

Soil pH and X-Ca were generally similar in all land use types, except for the Vegetable soils (Table 1), in which X-Ca was nearly three times higher than in any other land use type and soil pH tended toward neutrality. Similarly, X-Mg tended to be higher in these Vegetable soils, whereas little variation was observed in X-K between land use types.

Soils were moist due to recent precipitation prior to each sampling date. Grassland soils tended to have higher gravimetric moisture content than cultivated soils, but for a

given water content, soil matric potential was more negative in the grassland soils, as shown for the top 0–6 cm depth (Fig. 2). Therefore, mean soil matric potential for most of the sampling sites was ≥ -0.1 MPa.

Total soil C and N (Table 1) tended to be greater in grassland sites than in cultivated sites. The 9 land use types were condensed into four major groups to discern general trends. In increasing order, the sites were ranked as follows according to mean total soil C and N in the top 0–6 cm, respectively: non-irrigated agriculture (14.4 and 1.45 mg cm^{-3}), irrigated agriculture (15.7 and 1.55 mg cm^{-3}), perennial grasslands (24.6 and 2.35 mg cm^{-3}), and annual grasslands (24.9 and 2.48 mg cm^{-3}). Mean total soil C and N were similar between annual grasslands and perennial grasslands and between non-irrigated and irrigated agriculture (*t*-tests, $P \leq 0.05$, data not shown). Grasslands tended to have higher soil C and N in the 0–6 cm compared to the 6–12 cm layer.

Effects of land use type were evident in the microbial biomass, as measured by total PLFA, which was only sampled for the 0–6 cm layer (Table 1). Among the 9 land use types, total PLFA tended to be two to four times more abundant in grassland sites than cultivated sites. Again, this trend was more distinct when the four major groups were compared for total PLFA. In increasing order the sites were ranked as follows according to mean total PLFA: irrigated agriculture (10.6 μg g^{-1}), non-irrigated agriculture (13.4 μg g^{-1}), annual grasslands (28.3 μg g^{-1}), and perennial grasslands (40.5 μg g^{-1}). Total PLFA between irrigated and non-irrigated agriculture was similar. Total PLFA in perennial and annual grasslands differed from each other and from both agricultural groups (*t*-tests, $P \leq 0.5$, data not shown). There was a significant positive linear correlation of total PLFA with soil N ($r^2 = 0.54$), soil C ($r^2 = 0.57$), X-K ($r^2 = 0.12$), and percent plant cover ($r^2 = 0.27$) (data not shown). In contrast, total PLFA was negatively correlated with bulk density ($r^2 = 0.17$) and pH ($r^2 = 0.21$). The total PLFA was uncorrelated with any other variables (X-Ca, X-Mg, clay, sand, or silt content, or slope).

3.2. PLFA profiles

The CA of PLFA shows distinct clusters for most of the 9 land use types, as well as segregation of cultivated and grassland sites (Fig. 3). Microbial community composition, defined by the PLFA profile at each site, was therefore similar within a given land use type. Old fields with perennial grasses tend to be on the right of the origin of the biplot while the perennial grasslands that were never cultivated segregate to the left of the origin. Annual grasslands that were grazed and ungrazed are clustered in

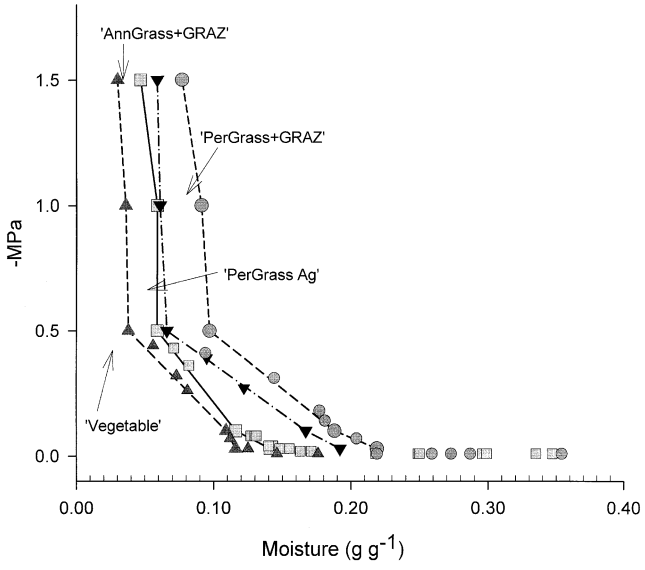

Fig. 2. Estimated mean soil matric potential for the 42 sites based on the soil moisture retention curve (SMRC) of the most closely corresponding land use type. For example, the SMRC for one of the soils was used to estimate soil matric potential for three land use types: AnnGrass + GRAZ = AnnGrass, AnnGrass + GRAZ and Hayfield. The large symbols connected by lines show the soil moisture retention curves for four of the management types. Small symbols indicate mean values of all 42 sampled land use types as follows: (●) Perennial grasslands, i.e. PerGrass Oldfield, PerGrass + GRAZ and PerGrass; (▼) PerGrass Ag; (▲) Cultivated sites, i.e. Vegetable and Fallow; (■) Annual grasslands, i.e. AnnGrass, and AnnGrass + GRAZ plus Hayfield.

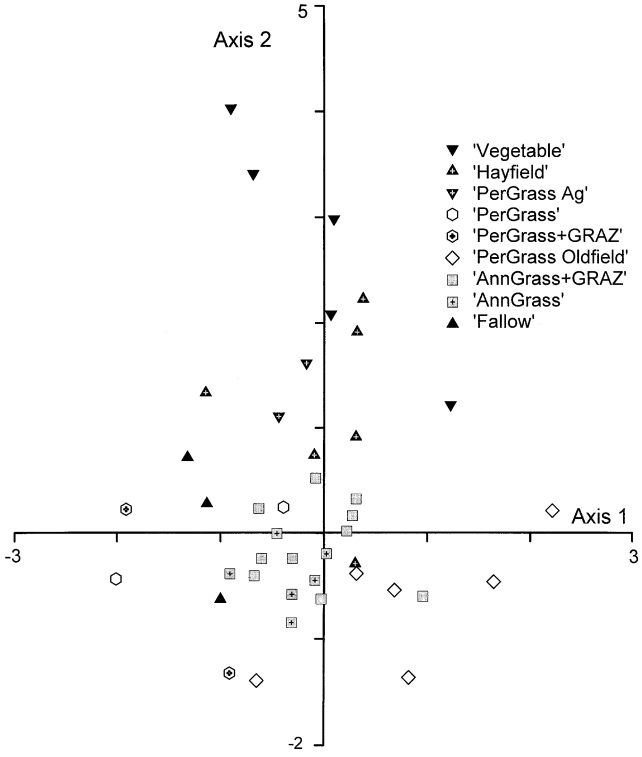

Fig. 3. CA ordination biplot of the PLFA profiles for the 42 surveyed sites, which are distinguished by the 9 land use types. Shorter distances between sites in the CA ordination indicate a greater degree of similarity between sites and their respective PLFA profiles. Axes 1 and 2 represent 47.1 and 20.4% of the variation in the data, respectively. See Table 2 for loading scores of individual PLFA.

the center of the biplot around the origin. Sites that had been in annual grassland, but were tilled for the previous 3 years (Fallow) with no plants present, sit to the left of the annual grassland sites. PLFA identified as i14:0, 18:2 ω6, and i15:1 tend to have greater abundances in PerGrass Oldfield sites (Table 2), while relatively greater abundances of 19 cy, 16:1 2OH, and 15:0 3OH are associated with PerGrass, PerGrass + GRAZ, and Fallow sites.

The CA biplot vertically separates cultivated sites from grasslands, with hay fields intermediate between intensively cultivated and grassland sites (Fig. 3). Perennial grass agricultural (PerGrass Ag) sites were located between the grassland sites and other cultivated sites, so that PLFA profiles bore more resemblance to cultivated than grassland sites. High positive scores for i17:1 and 18:3 ω6 indicate that they tend to have greater abundance in cultivated sites. Conversely, 18:2 ω6, 18:1 ω9, and 19:0 cy, exhibit the most negative scores along the vertical axis and have relatively greater concentrations in the grassland sites.

3.3. Relationship between PLFA profiles, soil characteristics, management and vegetation

Using canonical correlation analysis (CCA), we next identified the factors that best explain the pattern of PLFA profiles via Monte Carlo tests (Fig. 4; Table 2). Several factors related to cultivation (fertilizer, irrigation, herbicide, and time since last tillage event) significantly affect microbial community composition, as does grazing ($P \leq 0.05$; data not shown). Soil characteristics that have significant effects include total PLFA, and soil C and N, as well as clay, X-Mg, moisture content, and pH.

In the CCA biplot, perennial grasslands and old fields supporting perennial grasslands separate along the first axis (Fig. 4). Annual grasslands (grazed and ungrazed) cluster near the origin, and cultivated land use types are distributed in a positive direction along Axis 2. The clusters of land use types have a similar pattern as in the CA of PLFA (Fig. 3). Soil characteristics largely account for variation in microbial community composition of land use types distributed in relation to Axis 1 (Table 3). Microbial biomass (total PLFA) shows a positive association and relatively greater abundance in PerGrass Oldfield sites and some AnnGrass + GRAZ, while Clay, X-Mg, and moisture contents tend to have lower values and are negatively associated with these land use types. Thus, these four soil

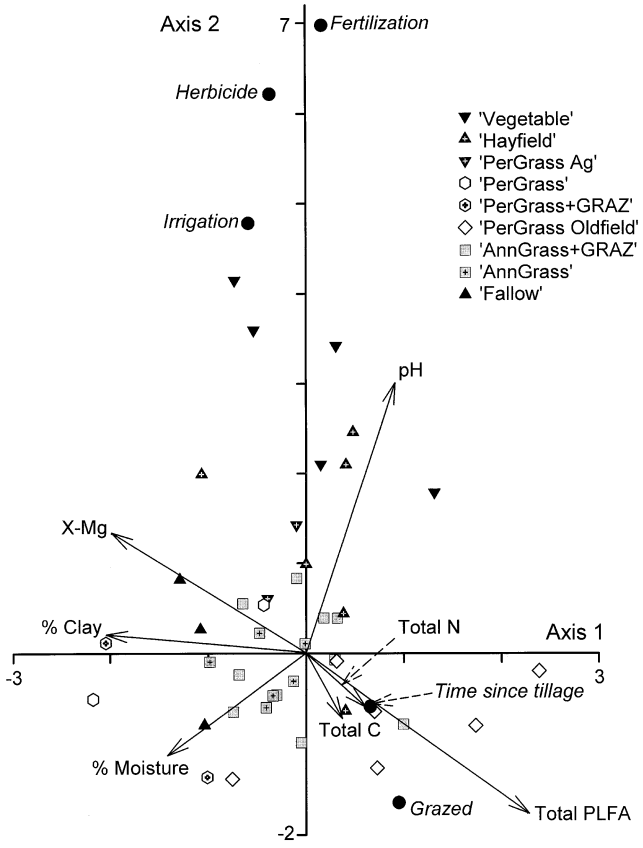

Fig. 4. CCA ordination biplot of the 42 sites, which are classified by the 9 land use types. Vectors represent the soil characteristics from 0 to 6 cm while centroids show the management factors that are significant by the Monte Carlo test ($P \leq 0.05$). Axes 1 and 2 represent 31.2 and 16.4% of the variation in the data, respectively. For biplot scores of soil characteristics and management factors, see Table 3, and for loading scores of individual PLFA, see Table 2. Biplot scores for soil characteristics and management factors are multiplied by a factor of four to provide greater clarity of presentation.

Table 2
Loading scores for the six PLFA of highest absolute value on Axes 1 and 2 of the biplots for the CA of PLFA (Fig. 3) and the CCA of soil characteristics from 0 to 6 cm depth, site and management factors and PLFA (Fig. 4)

Molecule	CA score	CCA score	Specificity as a marker
Axis 1			
16:1 2OH	−0.34	−0.32	None
19 cy[a]	−0.23	−0.21	Eubacterial anaerobes, Gram negative bacteria[b]
15:0 3OH	−0.19	−0.17	None
i15:1	0.18	0.16	Gram positive bacteria[b,c]
18:2 ω6	0.21	0.18	Fungi and eukaryotes[b]
i14:0	0.22	0.23	Gram positive bacteria[b]
Axis 2			
19:0 cy	−0.18	−0.13	Eubacterial anaerobes, Gram negative bacteria[d]
18:1 ω9	−0.10	−0.12	Fungi, plants, Gram positive bacteria[e]
18:2 ω6	−0.09	−0.07	Fungi and eukaryotes[b]
i17:1	0.15	0.16	Sulfate reducing bacteria, actinomycetes[d]
unknown	0.19	0.17	None
18:3 ω6	0.24	0.29	Fungi[d]

[a] This is an unresolved mixture of fatty acids containing a 19 carbon fatty acid with a cyclopropyl group.
[b] Federle (1986).
[c] Zelles (1997).
[d] Vestal and White (1989).
[e] O'Leary and Wilkinson (1988).

Table 3
Loading scores from multivariate analyses of CCA of soil characteristics, site and management factors and PLFA (Fig. 4)

Variable	Axis 1 (31.2% of variation)	Variable	Axis 2 (16.4% of variation)
Total PLFA ($\mu g\ g^{-1}$)	2.27	Fertilizer	6.97
Clay ($g\ g^{-1}$)	−2.03	pH	2.99
X-Mg ($meq\ 100\ cm^{-3}$)	−1.99	Herbicide	6.22
Moisture ($g\ g^{-1}$)	−1.41	Irrigation	4.78
Grazed	0.94	Total PLFA ($\mu g\ g^{-1}$)	−1.77

Soils data are from the 0–6 cm depth. Total variation explained by Axes 1 and 2, and the five values of highest absolute value for these two axes of the biplot are shown.

characteristics are associated with a gradient in microbial community composition that occurs among never-tilled perennial grasslands, annual grasslands, and old field perennial grasslands.

Changes in microbial community composition along the gradient of cultivated and grassland land use types are associated with both relatively lower microbial biomass (total PLFA) and higher soil pH, and management variables, including use of fertilizer, herbicide, and irrigation. Despite their significance in Monte Carlo tests, neither total soil C and N, nor time since the last tillage event are highly related to the gradients among grassland land use types (along Axis 1) or between cultivated and grassland land use types (along Axis 2), as indicated by their short vector lengths and wide angles of their positions with respect to the two axes. Analyzing the data with 'time since last tillage' as a covariate does not alter the amount of variation explained by the first two CCA axes or change the distribution patterns of sites or environmental variables on the biplot (data not shown). This, and the fact that Axes 1 and 2 together account for $<50\%$ of the variation in the data set, indicate that soil C and N, and the time since the last tillage event, play small but possibly complex roles in explaining PLFA profiles and microbial community composition.

4. Discussion

The diverse grassland and agroecosystems on granitic sandy loam soils in the central coast region of California could be distinguished from one another in terms of microbial community composition. Relict and restored stands of long-lived perennial bunchgrasses supported microbial communities that differed from those associated with formerly-cultivated annual grasslands, while intensive soil disturbance due to current cultivation distinguished the microbial community composition of agricultural sites from grasslands. Differences in microbial community composition were most highly correlated with soil microbial biomass (total PLFA) and pH, as well as with management factors such as fertilizer, herbicide, and irrigation.

4.1. Environmental factors and microbial community composition

In this study, PLFA profiles appear to be associated with factors that affect nutrient flux and turnover, or that may cause dynamic soil responses as a result of recent management inputs. For example, total PLFA, a measure of the soil microbial biomass, had large effects on distribution along both axes in the CCA biplot, whereas total organic C and N were not very important in explaining variation. Soil microbial biomass is considered to be part of the available or labile soil organic matter, the small fraction of the total soil organic matter that is readily decomposed and involved in nutrient cycling (Smith, 1979; Paul, 1984). Other studies also suggest that PLFA profiles or other measures of microbial community composition are clearly associated with the labile carbon fraction (Frostegård et al., 1997; Myers et al., 2001). Higher clay content typically is associated with increased labile soil organic matter because it provides greater adsorptive surface for interactions between the microbial biomass, soil organic matter, and the soil mineral fraction (Scow, 1997). Total PLFA would thus be expected to be highly correlated with clay content. A negative association between clay content and total PLFA, however, is observed along the gradient of grassland types in the CCA biplot. This suggests that clay may have an indirect effect on nutrient availability and microbial community composition, or, alternatively, that the unusually high clay content of two relict perennial grasslands (17 and 22%) affects the relative importance of the variable along this ordination axis. In any case, labile organic matter may play an important role in determining soil microbial community composition.

Our study suggests that non-native annual grasses may be associated with a unique microbial community: all sites that supported annual grassland had similar PLFA profiles, regardless of the time since the last tillage event occurred. Some sites were tilled as recently as 8 years ago, but others were last tilled more than 50 years ago. Plant community composition in annual grassland may have a rapid impact after abandonment of cultivated lands, and thereafter, display a consistent effect on microbial community composition. High productivity, dense accumulation of roots in the top 10 cm of soil, and complete die off of a plant community every year are conducive to building a large pool of active soil organic matter (Jackson et al., 1988) compared to cultivated sites (Syers, 1997). Furthermore, annual grassland is a plant community composed of less than a dozen dominant species of similar phenology in stands containing thousands of plants per square meter (Young et al., 1981). Although the group of dominant plant

species may change in relative abundance from year to year, plant community composition remains consistently similar with no succession towards a different community type (Heady, 1977; Pitt and Heady, 1978). Interestingly, when annual grassland sites were tilled for two consecutive years and had no plant cover during that time (Fallow), PLFA profiles diverged from the main cluster of annual grassland sites in the CA and CCA biplots (Figs. 2 and 3).

Unlike annual grasslands, wide variation in PLFA profiles occurred among the perennial grasslands (PerGrass + GRAZ, PerGrass, Pergrass Oldfield). *N. pulchra* was the only species sampled in never-tilled perennial grasslands (PerGrass + GRAZ and PerGrass), and other native and non-native bunchgrass species were sampled in the perennial grassland old fields (PerGrass Oldfield). Plant species identity may have been one factor for explaining the segregation of these three types of perennial grasslands. However, long-lived bunchgrasses may increase spatial heterogeneity in the soil (Hook et al., 1991) above that associated with annuals, and some perennial grass species have been found to influence the ratio of fungi to bacteria and to reduce the evenness of bacterial PLFAs (Bardgett et al., 1999b).

By taking an approach that surveys many sites on the same soil type but under diverse land use histories, it becomes apparent that multiple factors affect microbial community composition. Multivariate analysis provides a means to assess broad scale community structure, and generate hypotheses about the multiple controlling factors, i.e. pH, fertilizer, herbicide, and irrigation. Recent studies have shown that each of these factors individually affects microbial community composition. pH (Bååth et al., 1992; Frostegård et al., 1993; Bååth et al., 1995), fertilizer (Lovell et al., 1995), irrigation (Lundquist et al., 1999), and pesticide (Ka et al., 1995) are known to have immediate and pronounced effects on microbial biomass and community composition, and were shown here to have significant effects on PLFA profiles. Other studies have shown that PLFA profiles are influenced by management factors such as tillage (Petersen and Klug, 1994; Calderón et al., 2000; Calderón et al., 2001) and grazing (Frostegård et al., 1997; Bardgett et al., 1997), but they did not show clear effects on microbial community composition in this study.

4.2. PLFA as biomarkers

PLFA profiles represent the composition of the microbial community, yet they do not provide information about the identity of species within the community. Individual fatty acids cannot represent specific species of the microbial community because different species can share various fatty acids. Although PLFA analysis can identify large taxonomic groups of microbial organisms, problems can arise in ecological interpretation of community structure.

PLFA that were enriched in PerGrass Oldfield sites and some AnnGrass + GRAZ sites of both the CA and CCA (Figs. 2 and 3) were i14:0, 18:2 ω6, and i15:1, showing higher incidence of markers for gram positive bacteria, fungi and eukaryotes particularly in the old field perennial grassland sites. Associated with PerGrass + GRAZ and PerGrass, Fallow and some AnnGrass + GRAZ and AnnGrass were 19 cy, a biomarker for gram negative bacteria, and 16:1 2OH and 15:0 3OH, which have no known specificity. In the CA biplot, cultivated sites had higher concentrations of i17:1, a biomarker for sulfate reducing bacteria and actinomycetes, and 18:3 ω6, a fungal marker. In other studies, fungal biomarkers have been shown to decrease in response to simulated tillage (Petersen and Klug, 1994), but not in all cases (Calderón et al., 2000). Conversely, 18:2 ω6, 18:1 v9, and 19:0 cy tended to decrease with cultivation, indicating that several other bacterial and fungal groups were relatively more abundant in grassland soils. These findings are similar to those of field studies (Bardgett et al., 1999a), where fungal and bacterial biomarkers (18:2 ω6 and 19:0 cy) were more abundant in unfertilized grasslands than fertilized grasslands. In pot studies with homogenized soil, however, the 18:2 ω6 fungal biomarker increased in response to nutrient addition in bulk and rhizosphere soils (Steer and Harris, 2000). In our study, different fungal and bacterial biomarkers were more highly associated with either cultivated or grassland sites, suggesting that not all soil microorganism groups respond to a specific soil environment or management practice in the same manner. Soil microbial communities are unlikely to be discrete and fixed entities, i.e. the ‘organismic concept’, but rather assemblages of taxa that respond individually to variation in the environment, i.e. a more ‘individualistic concept’ (Kent and Coker, 1992).

Not all PLFA are easily categorized in terms of their association with environmental variables. For example, 19:0 cy is a fatty acid that is known to be a biomarker for anaerobic eubacteria (Vestal and White, 1989). It has also been found to be positively associated with high moisture content (Lundquist et al., 1999), low pH (Bååth et al., 1995) and root presence (Olsson et al., 1996), and negatively associated with high straw inputs (Bossio and Scow, 1998) and heavy metal pollution (Bååth et al., 1992). In our study, 19:0 cy was highly associated with grasslands, which were the wettest soils that we sampled. These sites also had the lowest pH, typically higher density of roots in the surface soil than in cultivated soils, and high litter inputs. When considering a combination of management factors, the directional effect of several different responses on 19:0 cy is difficult to discern.

4.3. Implications for ecosystem management

The PLFA profiles of perennial grass old field (PerGrass Oldfield) sites, which were tilled from 3 to 33 years ago, were distinctly different from those of relict grasslands (Figs. 3 and 4). Supporting the idea that recovery of the soil environment and the associated

microbial community from cultivation effects may require decades to centuries, Buckley and Schmidt (2001) found that the microbial community structure of an old field 7 years after abandonment from cultivation was still more similar to nearby cultivated sites than a never-cultivated field sharing a similar plant community. Likewise, the microbial community composition of PerGrass Ag sites that supported *N. pulchra* (i.e. the same species as in relict perennial grasslands) for 3 years prior to sampling is more similar to cultivated sites than the perennial grasslands. In contrast, annual grasslands show very similar microbial community compositions despite the variation in the time since tillage. More studies are necessary to understand the mechanism of this result, but it suggests that some aspect (e.g. plant composition and/or productivity) of annual grasslands influences the soil microbial community composition.

Finally, the identification of distinctive microbial community composition with a given land use type makes a compelling case for using PLFA profiles as 'fingerprints' for successful restoration of soil communities characteristic of native plant communities, or as indicators of changes in soil quality or responses to management inputs in agricultural systems.

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