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Variations in microbial community composition through two soil depth profiles

Noah Fierer[a,*], Joshua P. Schimel[a], Patricia A. Holden[b]

[a]*Department of Ecology, Evolution, and Marine Biology, University of California, Santa Barbara, CA 93106, USA*
[b]*Donald Bren School of Environmental Science and Management, University of California, Santa Barbara, CA 93106, USA*

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Abstract

Soil profiles are often many meters deep, but with the majority of studies in soil microbiology focusing exclusively on the soil surface, we know very little about the nature of the microbial communities inhabiting the deeper soil horizons. We used phospholipid fatty acid (PLFA) analysis to examine the vertical distribution of specific microbial groups and to identify the patterns of microbial abundance and community-level diversity within the soil profile. Samples were collected from the soil surface down to 2 m in depth from two unsaturated Mollisol profiles located near Santa Barbara, CA, USA. While the densities of microorganisms were generally one to two orders of magnitude lower in the deeper horizons of both profiles than at the soil surface, approximately 35% of the total quantity of microbial biomass found in the top 2 m of soil is found below a depth of 25 cm. Principal components analysis of the PLFA signatures indicates that the composition of the soil microbial communities changes significantly with soil depth. The differentiation of microbial communities within the two profiles coincides with an overall decline in microbial diversity. The number of individual PLFAs detected in soil samples decreased by about a third from the soil surface down to 2 m. The ratios of cyclopropyl/monoenoic precursors and total saturated/total monounsaturated fatty acids increased with soil depth, suggesting that the microbes inhabiting the deeper soil horizons are more carbon limited than surface-dwelling microbes. Using PLFAs as biomarkers, we show that Gram-positive bacteria and actinomycetes tended to increase in proportional abundance with increasing soil depth, while the abundances of Gram-negative bacteria, fungi, and protozoa were highest at the soil surface and substantially lower in the subsurface. The vertical distribution of these specific microbial groups can largely be attributed to the decline in carbon availability with soil depth.

Keywords: Microbial diversity; Phospholipid fatty acid; Soil profile; Community composition; Microbial biomass

1. Introduction

We know very little about the nature of the microbial communities found throughout the soil profile. Most studies in soil microbiology have focused exclusively on the surface 25 cm of soil where the densities of microorganisms are highest. However, soil profiles are often many meters deep and large numbers of microorganisms reside in subsurface horizons (Van Gestel et al., 1992; Dodds et al., 1996; Fritze et al., 2000; Blume et al., 2002). These subsurface microbes play an important role in soil formation, ecosystem biogeochemistry, contaminant degradation, and the maintenance of groundwater quality (Konopka and Turco, 1991; Hiebert and Bennett, 1992; Madsen, 1995; Richter and Markewitz, 1995), yet we know surprisingly little about the microbial communities residing in the deeper soil horizons.

Microbial community composition may be one important control on soil processes (Schimel, 1995; Cavigelli and Robertson, 2000; Balser et al., 2002). If the microbial communities residing at depth are simply diluted analogs of the surface microbial communities and exhibit minimal differentiation, the characteristics and properties of microbial processes should be fundamentally similar in the surface and subsurface horizons. However, deeper layers of soil may contain microbial communities that are specialized for their environment and fundamentally distinct from the surface communities (Ghiorse and Wilson, 1988; Zvyagintsev, 1994; Fritze et al., 2000; Blume et al., 2002). In this case, the microbial communities in the soil subsurface may function differently from those at the surface and their metabolic properties could not be inferred

* Corresponding author. Tel.: +1-805-893-5226; fax: +1-805-893-4724.
E-mail address: fierer@lifesci.ucsb.edu (N. Fierer).

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by studying the microbial communities found in the surface horizons.

We expect the strong resource and environmental gradients found within the soil profile to cause microbial community composition to change with soil depth. As we examine progressively deeper layers in the soil profile, the quantity and quality of C substrates decline, while soil moistures and temperatures become less variable. Surface soils are rich in available C substrates from the input of root exudates, surface litter and root detritus. In contrast, the rates of C input to the lower horizons are generally low and the C tends to be of limited lability (Richter and Markewitz, 1995; Ajwa et al., 1998; Trumbore, 2000). We would expect these changes in soil C availability to strongly influence the composition of the soil microbial communities (Zelles et al., 1995; Bossio and Scow, 1998; Griffiths et al., 1999). The soil surface experiences wider swings (both daily and seasonally) in soil temperatures and soil moisture than the soils found at greater depths (Brady and Weil, 2002). Both soil temperature (Zogg et al., 1997) and soil moisture (Kieft et al., 1993; Lundquist et al., 1999; Schimel et al., 1999) have been shown to influence soil microbial community composition. These gradients in resource availability and environmental stress are likely to be the primary factors controlling the nature and properties of the microbial communities residing in the soil profile.

The aim of this study was to determine the vertical distribution of microbial biomass and specific microbial populations in two soils from the Santa Ynez Valley, CA, USA. We wanted to know if surface microbial communities are fundamentally distinct from the communities inhabiting the deeper soil horizons and how the levels of diversity within microbial communities changes with soil depth. We used phospholipid fatty acid (PLFA) analysis as a tool to characterize the soil microbial communities found in samples collected from the soil surface down to 2 m in depth.

2. Methods

2.1. Profile characterization

The two soil profiles we studied are located on the University of California Sedgwick Reserve (34° 42′N, 120° 03′W), 30 miles north of Santa Barbara, CA in the Santa Ynez Valley. One profile was excavated on a Quaternary terrace site, where soils are relatively old but have formed in place with minimal erosional losses. This 'terrace' profile is classified as a Thermic Typic Argixeroll. Another profile was excavated on the valley floor, a depositional zone approximately 300 m down slope from the terrace site. This 'valley' profile is classified as a Thermic Pachic Haploxeroll. The parent material for both soil profiles is the Paso Robles formation, a weakly consolidated alluvium composed largely of shale deposited in the Pleistocene era (Dibblee, 1966). The vegetation on both sites is dominated by the annual grasses, Mediterranean barley (*Hordeum murinum* L.) and brome grass (*Bromus* spp.). The climate of the region is Mediterranean with an average annual rainfall of 50 cm with almost all of this rainfall occurring between the months of December and April. Selected physical and chemical characteristics of the two profiles are shown in Table 1. Compared to the terrace, the valley profile contains more organic matter, is slightly more basic, and is generally more moist throughout the year.

2.2. Soil sampling

The valley and terrace profiles were excavated in April and October of 2000, respectively. The resulting trenches measured 10 m × 2 m with depths of 4 m in the valley site and 3 m in the terrace site. Immediately after excavation, the trench walls were sealed with an opaque plastic vapor barrier. Both trenches were roofed and insulated. The trench

Table 1
General physical and chemical characteristics of the soil profiles at the two study sites. Average temperatures and temperature ranges obtained from data collected between March 2001 and September 2001. Moisture content data (% of dry weight) were obtained from 4 sampling times (Fall 2000, Winter 2001, late Spring 2001, and late summer 2001). One standard error reported in parentheses. $N = 3$ for each sampling depth

Profile	Sampling depth (cm)	pH	% C	% N	Moisture content at time of sampling (%)	Texture	Horizon	Average temperature (°C)	Range in temperatures (°C)	Average annual moisture content (%)	Range in moisture contents (%)
Terrace	0–5	5.5	2.6 (0.37)	0.29 (0.028)	29.6 (2.91)	Loam	A	25	2.0–60	9.2	0.61–30
	5–15	5.5	1.7 (0.17)	0.23 (0.013)	22.7 (0.632)	Loam	A	27	18–44	8.0	2.2–25
	15–25	5.5	1.2 (0.075)	0.18 (0.0061)	21.4 (1.24)	Loam	A	26	13–36	9.4	4.9–27
	50	5.4	0.46 (0.022)	0.12 (0.0058)	5.70 (0.813)	Loam	B	22	14–28	11	5.7–29
	100	6.2	0.16 (0.0042)	0.10 (0.0038)	14.6 (0.502)	Clay loam	B	20	14–25	10	15–24
	200	6.7	0.10 (0.0074)	0.08 (0.018)	16.7 (0.958)	Sandy loam	BC	19	14–24	10	17–19
Valley	0–5	6.1	3.2 (0.26)	0.30 (0.024)	35.4 (1.26)	Loam	A	24	3.5–53	11	5.8–35
	5–15	6.2	2.0 (0.068)	0.21 (0.011)	27.4 (1.24)	Loam	A	25	8.9–40	10	4.4–29
	15–25	6.2	1.7 (0.020)	0.18 (0.0016)	24.8 (0.382)	Loam	A	25	12–32	11	8.4–29
	50	6.7	0.83 (0.019)	0.10 (0.0022)	23.8 (5.32)	Loam	AB	22	13–26	14	18–30
	100	6.9	0.84 (0.022)	0.10 (0.0031)	22.4 (4.95)	Clay loam	B	20	14–23	14	19–30
	200	7.5	0.16 (0.024)	0.050 (0.0022)	23.7 (0.575)	Clay loam	B	18	14–22	16	24–31

structures were designed to permit repeated sampling of the soil profile in a natural, undisturbed, state. The soil sampling was conducted in October 2000. The first measurable rain event in 4 months occurred 1 week prior to sampling so the surface soils were slightly moist (Table 1). The depth increments for soil sampling are shown in Table 1. Three replicate soil samples were collected from each profile at the surface depth increments (0–5, 5–15, and 15–25 cm) by digging vertically from the surface at three randomly chosen locations within 5 m of the trenches. The deeper soil samples were collected by horizontally coring from the inner walls of the trenches. The outer 25 cm of each core (the 25 cm closest to the trench wall) was discarded from each sample to ensure that the collected soil was not affected by the presence of the excavated trench. Three replicate samples were taken at each depth from randomly chosen locations within each trench. The samples were immediately transported back to the laboratory, sieved to 4 mm, homogenized, and stored at 4 °C. All visible root and fresh litter material were removed from samples prior to sieving. Care was taken to prevent cross-contamination of the soil samples during and after collection.

2.3. Chemical and physical soil characteristics

The soil samples were analyzed for a variety of physical and chemical characteristics to determine the gradients in pH, organic matter, texture, and moisture content through both profiles (Table 1). Total carbon and nitrogen content was determined using a Fisons NA1500 C/N analyzer. Soil inorganic C ($CaCO_3$) concentrations were determined by standard methods (Allison and Moodie, 1965) with CO_2 measured using an infrared gas analyzer (Licor Model LI-6252). Organic C was calculated by subtracting the inorganic C from the total soil C. Soil pH was measured with a pH meter (Corning Model 320) after equilibrating 15 g of dry soil with 15 ml of deionized water for 30 min. Particle size analysis was conducted at the Division of Agriculture and Natural Resources Analytical Laboratory, University of California Cooperative Extension (Davis, CA). Gravimetric soil moisture content was determined by the mass difference before and after drying at 120 °C for 48 h. Horizon designations were assigned using field and laboratory data in accordance with the USDA soils classification scheme (Staff, 1996).

The variability in soil temperature regimes through the profiles was measured using two Type K thermocouples permanently installed in the trench walls at each sampling depth. Temperature averages were automatically logged every 2 h between March 2001 and September 2001 with a Campbell CR10X (Logan, UT) datalogger. Annual variability in soil moisture was assessed with soil samples collected from the profiles in October, March, May, and September of 2000–2001.

2.4. Phospholipid fatty acid analysis

PLFA analysis was conducted on freeze dried soil samples using previously described procedures (White and Ringelberg, 1998). PLFAs were extracted from 5 g of freeze dried soil for the 0–5 cm depths, from 10 g for the 5–25 cm depths, and from 20 g for the 50–200 cm depths in order to compensate for the decrease in microbial biomass with depth. A total of three samples were analyzed per depth within each profile. Extracted PLFA samples were analyzed using a Hewlett Packard 6890 Gas Chromatograph as previously described (Waldrop et al., 2000). For each sample, equivalent amounts of total extracted PLFAs were analyzed on the GC to assure that the decrease in microbial biomass with soil depth did not affect our characterization of the microbial communities through the soil profile. PLFA peaks were tentatively identified using bacterial fatty acid standards and MIDI peak identification software (MIDI, Inc., Newark, DE). PLFAs are designated using the nomenclature described in Frostegard et al. (1993).

Individual PLFA markers were used to quantify the relative abundances of specific cell types through the two soil profiles. Gram-positive bacteria were identified by the PLFAs: i14:0, i15:0, a15:0, i16:0, i17:0, a17:0 with Gram-negative bacteria identified by the PLFAs: cy17:0, cy19:0, 16:1ω9c, 18:1ω9c, 15:1ω4c, 18:1ω7c, 17:1ω9c (O'Leary and Wilkinson, 1988; Zelles et al., 1994; White et al., 1996; Zelles, 1997). Other microbial groups identified with PLFA biomarkers include fungi (18:2ω6c, (Frostegard et al., 1993; Zelles, 1997)), protozoa (20:2ω6,9,c, 20:3ω6,9,12c, 20:4ω6,9,12,15c (White et al., 1996)) and actinomycetes (10Me16:0, 10Me17:0, 10Me18:0, (Kroppenstedt, 1985; Brennan, 1988; Zelles et al., 1994)) Since the use of PLFAs as biomarkers to identify specific microbial types can be problematic, particularly in environmental samples containing diverse microorganisms, the proposed microbial groupings should be considered approximate (Zelles, 1997). The following two ratios of PLFA relative abundances were also calculated/cyclopropyl fatty acids/monoenoic precursors (cy17:0 + cy19:0/16:1ω7c + 18:1ω7c) and total saturated/total monounsaturated fatty acids (12:0 + 13:0 + 14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0/14:1ω5c + 15:1ω6c + 16:1ω7c + 16:1ω5c + 17:1ω9c + 18:1ω9c + 18:1ω7c). Both of these ratios have previously been used as indicators of nutritional stress in bacterial communities (Knivett and Cullen, 1965; Kieft et al., 1997; Bossio and Scow, 1998).

2.5. Microbial biomass measurements

We estimated total microbial biomass within the profiles using three separate metrics: total extractable PLFAs, substrate-induced respiration (SIR), and chloroform fumigation–extraction. The sum of the total extractable PLFAs from each soil sample has previously been shown to be

proportional to other microbial biomass measures (Balkwill et al., 1988; Fritze et al., 2000). Both PLFA analysis and the fumigation–extraction technique estimate the size of the microbial biomass pool without regard to the physiological status of the microbes, the SIR method preferentially measures that portion of the microbial community capable of rapid respiration with an easily degradable carbon substrate (Wardle and Ghani, 1995). The chloroform fumigation–extraction method we used to measure chloroform-extractable biomass C is described in Fierer and Schimel (2002). The SIR method was modified from that described in West and Sparling (1986). Soil samples (10 g, dry weight equivalent) were weighed into individual 50 ml centrifuge tubes and 10 ml of yeast extract solution (12 g autolyzed yeast, Difco Laboratories, Detroit, MI, in 1 l H_2O) was added to each tube. The tubes were sealed with gas-tight lids fitted with rubber septa and placed horizontally on a shaker (100 rpm) for the duration of the 4 h incubation. At 0, 2, and 4 h, cumulative CO_2 concentrations were measured inside each tube with an infrared gas analyzer (Licor Model LI-6252). The average respiration rate (μg C-CO_2 g $soil^{-1}$ h^{-1}) over the entire incubation period serves as an index of the SIR-responsive microbial biomass pool.

2.6. Statistical analyses

The concentrations of individual PLFAs, calculated as the mole percentage of the total area of the chromatogram, were used as input values in principal components analysis (PCA), to determine if the PLFA signatures of microbial communities varied with soil depth. Using PCA, we can combine the 90-dimensional data (a total of 90 different PLFAs were identified from all the samples) into a series of linear axes that incorporate the maximum amount of variance in the data. PCA was conducted using Systat software (Systat, 2000). The factor loading scores for the individual PLFAs were used to assess the relative importance of each individual PLFA in the calculation of the principal component axes. analysis of variance (ANOVA) procedures, with Fisher's LSD tests as post-hoc tests (Systat, 2000), were used to test for significant differences in microbial community composition and structure with soil depth.

3. Results

Total microbial biomass declined substantially with depth in both profiles (Table 2). The change in microbial biomass with soil depth was significant in both profiles and with all three of the metrics used to estimate microbial biomass ($P < 0.001$ in all cases). By all three methods, the valley profile had significantly higher microbial biomass densities in the top 5 cm of soil than the terrace profile ($P < 0.02$ in all cases). Microbial biomass estimated by summing PLFAs was well correlated with total chloroform-extractable biomass C across the range of values (Fig. 1(a)). The linear regression equations describing the relationship between the two methods are similar for both the 'high biomass' samples (0–5 cm depth) and the 'lower biomass' samples (below 5 cm depth). This in contrast to the comparison of biomass estimates by SIR and total extractable PLFAs (Fig. 1(b)) where the linear regression equation for the 0–5 cm samples is different than the equation describing the biomass estimates for samples below 5 cm. The relative decrease in estimated microbial biomass with depth was larger for the SIR-responsive biomass pools than for the biomass estimates obtained by summing extractable PLFAs.

The number of detectable PLFAs extracted from the soil samples (PLFA 'richness') decreased with soil depth (Fig. 2). Approximately 60 different PLFAs were extracted

Table 2
Microbial abundances within the two soil profiles, as estimated by SIR, summing extractable PLFAs, and chloroform fumigation-extraction. One standard error in parantheses, $N = 3$ for each sampling depth

Profile	Sampling depth (cm)	SIR rate, (μg C-CO_2 g $soil^{-1}$ h^{-1})	Microbial biomass by PLFA (nmol PLFA g $soil^{-1}$)	$CHCl_3$-extractable microbial biomass C (μg C g $soil^{-1}$)
Terrace	0–5	42 (7.0)	9.8 (1.6)	359 (17.3)
	5–15	9.9 (0.69)	4.0 (0.16)	262 (26.7)
	15–25	2.3 (0.033)	2.0 (0.12)	140 (7.44)
	50	0.60 (0.13)	0.63 (0.044)	55.6 (26.0)
	100	0.22 (0.021)	0.18 (0.030)	11.9 (4.99)
	200	0.24 (0.052)	0.081 (0.0053)	12.1 (5.64)
Valley	0–5	55 (1.7)	16 (0.040)	676 (30.0)
	5–15	10 (0.12)	5.1 (0.41)	209 (19.1)
	15–25	2.8 (0.41)	2.5 (0.16)	73.9 (10.2)
	50	0.89 (0.15)	0.84 (0.077)	57.7 (23.0)
	100	0.67 (0.26)	0.41 (0.093)	79.0 (22.4)
	200	0.19 (0.025)	0.11 (0.043)	24.7 (20.2)

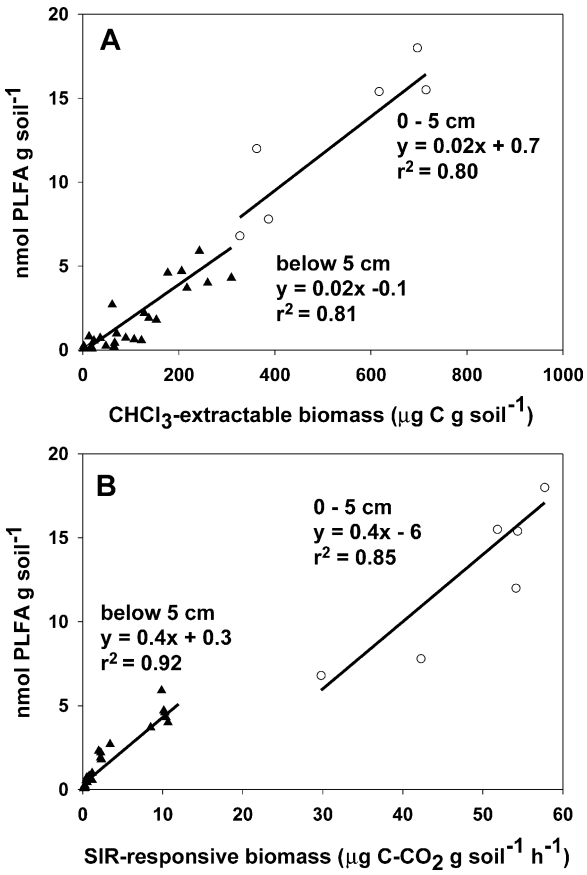

Fig. 1. The relationships between total microbial biomass estimates obtained by summing extractable PLFAs and the biomass estimates obtained by chloroform fumigation extraction (A) and SIR (B) for all samples. Separate regression lines have been drawn for the high biomass samples collected from the 0–5 cm depth and the lower biomass samples from the 5–200 cm depths.

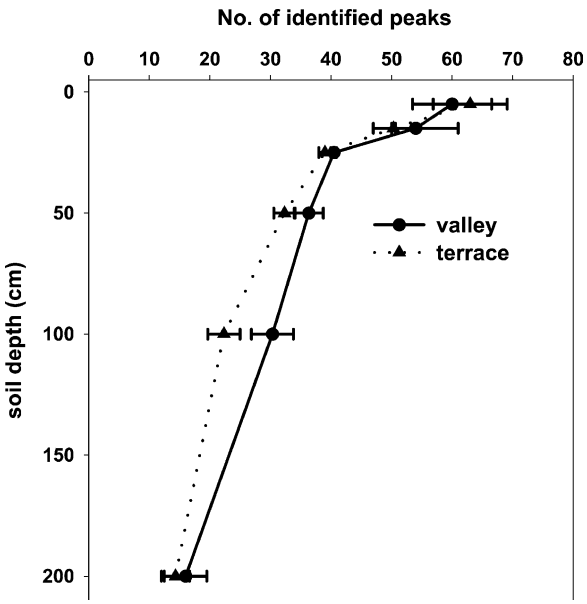

Fig. 2. The number of detectable PLFAs extracted from the soil samples (PLFA richness) with soil depth in the two profiles. Error bars = 1 SE, $N = 3$.

from the 0–5 cm soil depths while only 15 PLFAs were extracted from the 2 m depths. The two profiles exhibited very similar patterns of PLFA richness with depth, although slightly more PLFAs were extracted from the 1 m depth in the valley profile than in the terrace profile.

PCA of the PLFA data suggests a substantial level of differentiation in microbial community structure with soil depth (Fig. 3). The first principal component axis (PC1) explained 42% of the variance in the data while the second principal component axis (PC2) explained only 12%. The relationship between the PC1 scores and soil depth was statistically significant in the two profiles ($P < 0.0001$ in both cases). The degree of differentiation in microbial communities with soil depth is almost identical for the two profiles examined. Overall, the distinctiveness of the PLFA signatures by depth is greater than the differences between samples collected at the same depth in the two different soil profiles. The variance in the PLFA signatures from replicate samples is higher at the soil surface compared to samples from greater depths.

The differences in PLFA signatures with soil depth are primarily a result of individual PLFAs decreasing in proportional abundance with depth. The majority of the 90 PLFAs had positive factor loading scores on PC1, indicating a decrease in proportional abundances with depth. The individual PLFAs with the highest positive loading scores in both profiles are shown in Table 3. Only 21 and 18 PLFAs in the valley and terrace profiles, respectively, appeared to become proportionally more abundant with depth, i.e. had negative PCA loading scores. The PLFAs that increased substantially in proportional abundance with depth (loading score < -0.2) in both profiles are identified in Table 3.

Using specific PLFAs as biomarkers (see Section 2), we can quantify the relative abundances of specific microbial groups within the soil profiles (Table 4). In both profiles,

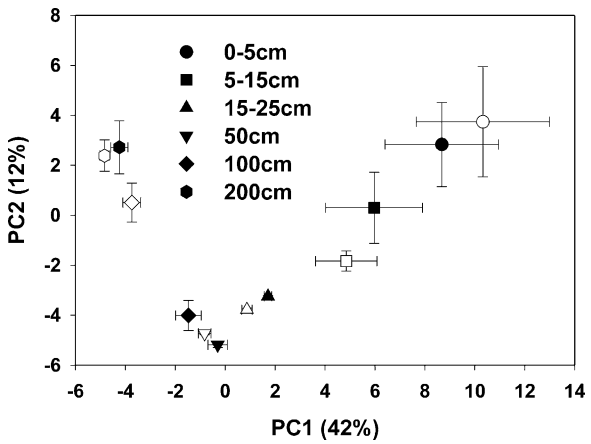

Fig. 3. Principal component analysis (PCA) of PLFA signatures (mol percentages) from soil samples collected at different depths within the two profiles. The same symbols are used for the different depth increments in the valley and terrace profiles. The valley profile samples are represented by the filled symbols. PC1 explains 42% of the variance in the PLFA data, PC2 explains 12%, the individual scores are unit less. Error bars indicate ± 1 SE ($N = 3$).

Table 3
Individual PLFAs most responsible for the changes in PLFA signatures with sample depth along PC1. A positive loading score is driven by a decrease in proportional abundance with depth, a negative loading score indicates an increase in proportional abundance. We selected the PLFAs with loading scores that were relatively high in magnitude and had similar scores in both profiles. PLFAs belonging to notional microbial groups (see Section 2) are identified as follows

PLFA	Loading score in valley profile	Loading score in terrace profile
18:1	0.93	0.83
20:4ω6,9,12,15c[a]	0.91	0.86
20:3ω6,9,12c[a]	0.83	0.86
a15:1	0.90	0.89
18:2ω6c[b]	0.86	0.94
i15:1	0.84	0.93
16:1ω7c	0.83	0.91
i15:0[c]	0.81	0.90
i13:0	0.85	0.88
16:1ω5c	0.84	0.86
18:0	−0.52	−0.70
i17:1ω7c	−0.38	−0.59
10Me16:0[d]	−0.75	−0.43
16:1	−0.59	−0.44
cy19:0[e]	−0.55	−0.21
i16:1	−0.53	−0.29
a15:0[c]	−0.38	−0.40

[a] Protozoa.
[b] Fungi.
[c] Gram-positive bacteria.
[d] Actinomycetes.
[e] Gram-negative bacteria.

the proportion of the total PLFAs attributable to fungi was higher at the soil surface than at deeper depths. The abundance of fungi was significantly related to soil depth in the valley profile, but not in the terrace profile ($P = 0.003$ and 0.20, respectively). The proportional abundances of Gram-positive bacteria increased with depth, while the proportional abundances of Gram-negative bacteria generally declined with soil depth. The distributions of both Gram-negative and Gram-positive bacteria were significantly related to soil depth in the two profiles ($P < 0.04$ in all cases). Specific PLFAs indicative of protozoa were detected in the upper horizons of both profiles but not at greater depths. The proportional abundances of actinomycetes were the lowest in the top 5 cm of the profiles and generally increased at greater soil depths. Actinomycete abundances were only significantly related to soil depth at the terrace site ($P = 0.01$ and 0.07 in the terrace and valley profiles, respectively).

The two calculated PLFA ratios, cyclopropyl fatty acids/monoenoic precursors and total saturated/total monounsaturated fatty acids, increased with depth in the two profiles (Table 4). The two ratios were significantly related to soil depth in both the terrace and valley profiles ($P < 0.04$ in all cases).

4. Discussion

4.1. Overall patterns in biomass and microbial diversity with soil depth

All of the methods we used to estimate microbial biomass indicate that microbial densities are one to two orders of magnitude higher at the soil surface than at 2 m depth. Similar patterns in microbial abundances have been observed within other soil profiles (Federle et al., 1986; Kaiser and Heinemeyer, 1993; Dodds et al., 1996; Murphy et al., 1998; Taylor et al., 2002). The microbes inhabiting the deeper soil horizons have generally been considered unimportant because of the low biomass densities and the low levels of activity. If we assume a constant bulk density (1.5 g cm^{-3}) through the profiles and use the microbial

Table 4
The distribution of notional microbial groups, as identified using PLFA biomarkers, through the two soil profiles. Also shown are two PLFA ratios which may be indicative of the nutritional status of microbial communities. See Section 2 for identification of the PLFAs used to calculate the abundances of the specific microbial groups and the two ratios. One standard error indicated in parentheses, $N = 3$ in all cases

Profile	Sampling depth (cm)	Gram-positive bacteria (mol%)	Gram-negative bacteria (mol%)	Actinomycetes (mol%)	Protozoa (mol%)	Fungi (mol%)	cy17:0, cy19:0: precursors	Total saturated: total monounsaturated
Terrace	0–5	22 (0.95)	24 (0.98)	4.7 (1.4)	0.70 (0.026)	2.9 (0.16)	0.27 (0.0059)	0.61 (0.013)
	5–15	25 (0.59)	20 (0.29)	11 (0.082)	0.62 (0.086)	1.8 (0.36)	0.40 (0.0035)	0.74 (0.014)
	15–25	27 (0.40)	18 (0.33)	12 (0.46)	0.50 (0.15)	1.1 (0.074)	0.55 (0.036)	0.93 (0.017)
	50	32 (0.88)	13 (0.11)	17 (1.2)	0[a]	1.1 (0.21)	1.1 (0.033)	1.4 (0.029)
	100	24 (4.1)	13 (3.6)	13 (0.86)	0[a]	1.4 (0.33)	1.4 (0.37)	2.5 (1.1)
	200	28 (3.2)	5.6 (0.44)	10 (2.1)	0[a]	0[a]	–[a]	3.6 (0.54)
Valley	0–5	18 (1.0)	29 (0.59)	8.4 (0.17)	0.78 (0.10)	3.6 (0.25)	0.16 (0.017)	0.42 (0.0088)
	5–15	22 (0.29)	21 (0.67)	12 (0.17)	0.81 (0.058)	1.8 (0.40)	0.31 (0.013)	0.62 (0.016)
	15–25	24 (0.50)	18 (1.0)	14 (0.087)	0.46 (0.018)	1.2 (0.022)	0.43 (0.026)	0.85 (0.035)
	50	28 (0.48)	13 (0.18)	16 (1.1)	0[a]	1.4 (0.33)	0.74 (0.11)	1.1 (0.081)
	100	26 (0.61)	14 (0.57)	16 (0.73)	0[a]	1.8 (0.056)	0.84 (0.13)	1.2 (0.12)
	200	26 (3.6)	14 (2.5)	11 (0.34)	0[a]	0[a]	0.50 (0.25)	1.3 (0.078)

[a] Mol% below the detection limit of the instrument.

biomass estimates obtained by summing extractable PLFAs (Table 2), we calculate that approximately 35 and 33% of the microbial biomass in 2 m of soil is below 25 cm in depth in the valley and terrace profiles, respectively. Not only do significant numbers of microbes reside in the soil subsurface, but the potential activity of these subsurface microbes (on a per cell basis) can be as high or higher than microbes at the surface (Tate, 1979; Federle, 1988; Blume et al., 2002).

In recent years, ecologists have devoted a considerable amount of effort attempting to describe the general relationship between ecosystem productivity, or total biomass, and species richness (see review by Mittelbach et al. (2001)). The observed patterns relating site productivity, or biomass, and species richness generally fall into two categories: a 'hump-backed' relationship, where species richness peaks at intermediate levels of productivity, or a broad positive relationship, where higher levels of species richness are found in more productive and more biomass-rich sites (Waide et al., 1999; Mittelbach et al., 2001). As far as we know, all of the published studies relating species richness to site productivity have focused on the richness of macroorganisms. The soil profile, with its strong gradient in productivity (as evidenced by the decline in microbial biomass with depth), provides a unique system in which microorganisms can be used to test these competing diversity–productivity relationships. In the two soil profiles studied, the PLFA richness (the number of individual PLFAs extracted from soil) and the total number of extracted PLFAs (microbial abundance) decreased with depth. Federle et al. (1986) have observed similar reductions in both PLFA richness and overall PLFA abundance within four soil profiles (three Ultisols and an Alfisol) sampled from Alabama, USA. If we consider PLFA richness to be an indicator of overall microbial community richness, we find that both studies agree with the later category, there is a broad positive relationship between microbial community richness and microbial biomass within soil profiles.

4.2. Comparisons with related studies

In both of the profiles we examined, the composition and structure of soil microbial communities changed significantly with soil depth. A number of other studies have also found that subsurface microbial communities are distinct in composition from the surface communities (Ghiorse and Wilson, 1988; Zvyagintsev, 1994; Fritze et al., 2000; Blume et al., 2002). The proportional abundances of fungi and protozoa decline with depth in the two profiles described here (Table 4) and in other soil profiles (Sinclair and Ghiorse, 1987; Richter and Markewitz, 1995; Strauss and Dodds, 1997; Ekelund et al., 2001; Taylor et al., 2002). The observed increase in the proportional abundances of actinomycetes with depth (Table 4) parallels results from other studies that have detected relatively large actinomycete populations in deeper soil horizons (Federle et al., 1986; Fritze et al., 2000) and in deep vadose zone sediments (Haldeman and Amy, 1993). The vertical distribution of Gram-negative and Gram-positive bacteria within the two soil profiles (Table 4) are consistent with the patterns observed in other soil profiles where microbial communities generally shift from greater Gram-negative dominance at the soil surface to greater Gram-positive dominance at deeper soil depths (Franzmann et al., 1998; Blume et al., 2002).

By comparing our study with the small number of comparable studies, we can tentatively suggest that the vertical distribution of broadly-defined microbial groups is similar among a wide range of soil types. However, one limitation of using the PLFA method to examine microbial community structure is that it can be difficult to accurately link the observed changes in PLFA patterns to the dynamics of specific groups of microorganisms. The application of DNA or RNA-based methods of community analysis will enhance our ability to understand the distribution of taxonomically-relevant groups of microbes in these and in other soil profiles.

4.3. Why do the microbial communities change with soil depth?

We hypothesize that soil resource availability is the main factor responsible for the observed changes in microbial community composition through the soil profiles. Soil pH, temperature, and texture are not likely to cause differentiation of microbial communities within the soil profiles studied. The nature of the microbial communities changes substantially within the top 50 cm of both profiles, yet soil pH, soil texture, and average annual soil temperatures are relatively constant within this range (Table 1). The climate at the study site is relatively mild, only at the soil surface are temperatures likely to be extreme enough to significantly influence microbial community composition, below the soil surface the ranges in measured soil temperatures are well within the physiological limits of mesophilic bacteria and fungi (Table 1, (Madigan et al., 1997)). We would also not expect soil O_2 to be an important factor causing the differentiation of the microbial communities within the studied profiles; there is no pedological evidence for recurrent anaerobiosis in either profile and the soils at these sites are rarely saturated with water, even in years of high rainfall (F. Chamran, 2000, PhD thesis, University of California).

In the semi-arid environment of the research site, soil water content may be partially responsible for the differentiation of microbial communities with soil depth. While average annual soil moisture does not vary appreciably within the top 2 m of the soil profile, the variability in soil moisture is much higher at the soil surface than at greater depths (Table 1). Previous work has shown that variability in soil moisture can influence the composition of soil bacterial and fungal communities (Schimel et al., 1999; Wilkinson et al., 2002). In these profiles,

moisture variability is only likely to be an important control on microbial community composition at the very surface of the soil profile where large shifts in water content are frequent and rapid enough to select for individual microbial groups with a high tolerance for moisture stress (Harris, 1981).

Additional factors which are not directly linked to the specific environmental conditions found within the soil profiles may also contribute to the differentiation of microbial communities. These factors may include: the selective transport of microbes through the soil profile by water or bioturbation (Abu-Ashour et al., 1994; Balkwill et al., 1998; Kieft et al., 1998) or the presence of microbes in deeper soil horizons that have been derived from the original depositional environment and have persisted in the soil for extended periods of time (Kennedy et al., 1994; Kieft et al., 1998). With this study, we cannot effectively assess the importance of these factors so we will limit our discussion to the environmental factor most likely to be responsible for the specific changes in microbial community composition with soil depth: soil resource availability.

The decline in resource availability through the soil profile is predominately a function of both decreasing carbon concentrations (Table 1) and a reduction in carbon quality with soil depth (Richter and Markewitz, 1995; Ajwa et al., 1998; Trumbore, 2000; N. Fierer, unpublished data). By comparing the microbial biomass estimates obtained by the three separate methods (Fig. 1) we can examine the influence of this gradient in C availability on the nature of the microbial communities within the two soil profiles. The PLFA extraction and chloroform fumigation-extraction methods estimate microbial biomass by exposing cells to organic solvents and quantifying extracted cell constituents. These two methods are indirectly measuring the same pool of microbial biomass, so we would expect the estimates of microbial biomass by the two methods to be linearly correlated across the full range of soil depths (Fig. 1(a)). In contrast, a comparison of the biomass estimates obtained by the SIR method and the PLFA method (Fig. 1(b)) shows that the SIR method tends to overestimate (relatively) the microbial biomass at the soil surface (0–5 cm depth) and to underestimate biomass in the deeper horizons. The SIR method preferentially measures the fast-growing, zymogenous portion of the microbial community since microbial biomass is assumed to be proportional to the short-term respiration rates measured after the addition of a labile substrate to a soil sample (Wardle and Ghani, 1995). We can therefore surmise that, compared to the subsurface microbial communities, a higher proportion of the microbes inhabiting the soil surface are physiologically or phylogenetically pre-adapted for rapid metabolism of labile carbon substrates. Since the rooting zone of annual grasses at this site is primarily within the top 10 cm of the soil profile (N. Fierer, personal observation), we would expect the high availability of root exudates and/or fresh litter at the soil surface to select for microbial communities that are able to rapidly utilize these labile carbon substrates.

The two PLFA ratios which have been linked to nutritional stress in microorganisms, cyclopropyl fatty acids/cyclopropyl precursors and total saturated/total monounsaturated fatty acids (Table 4), shift in accordance with the vertical gradient in C availability. A higher cyclopropyl to cyclopropyl precursor ratio has been associated with a decrease in bacterial growth rates and an increase in carbon limitation (Knivett and Cullen, 1965; Guckert et al., 1986; Kieft et al., 1997; Bossio and Scow, 1998). Similarly, the ratio of total saturated/total monounsaturated fatty acids is generally higher in microbial communities that inhabit environments where organic carbon and/or nutrients are limiting (Zelles et al., 1992; Kieft et al., 1997; Bossio and Scow, 1998; Lundquist et al., 1999). In both profiles, these two fatty acid ratios increased with soil depth, suggesting that the microbial communities residing in the deeper soil horizons are more severely resource limited than their surface-dwelling counterparts.

Given results from other studies, there are several predictions we might make about the vertical distribution of specific microbial groups solely on the basis of this decline in resource (carbon) availability with soil depth. Griffiths et al. (1999) have shown that higher rates of C addition to soil will raise the proportions of fungi and Gram-negative bacteria (cy17:0) in the microbial community and lower the proportions of actinomycetes and Gram-positive bacteria. These results would predict (accurately) that the proportional abundances of actinomycetes and Gram-positive bacteria should increase with soil depth while the abundances of fungi and Gram-negative bacteria should decrease (Table 4). We would also expect protozoan abundances to be positively correlated with resource availability and to decrease with soil depth since protozoa generally graze on bacteria (Stout, 1980) and the density of protozoa in a given soil sample should be indirectly proportional to the rates of bacterial growth (Sinclair and Ghiorse, 1987; Madsen et al., 1991). This is confirmed by studies showing higher protozoan densities in unsaturated sediments enriched in organic carbon contaminants compared to adjacent pristine sites (Madsen et al., 1991; Sinclair et al., 1993).

The two profiles we studied are similar in composition and are located in close proximity to one another. The valley profile, with its higher organic C concentrations (Table 1) and greater microbial densities (Table 2), is likely to have more available C than the terrace profile. The PLFA signatures of the microbial communities residing in the two soil profiles reflect this difference in resource availability. At corresponding soil depths, the valley profile generally has lower values for the fatty acid ratios indicative of nutrient stress and higher proportional abundances of Gram-negative bacteria, fungi, and protozoa than the terrace profile (Table 4).

Differences in soil C availability result in predictable and consistent changes in the properties of microbial communities. These changes are evident within individual soil depth profiles and, to a lesser extent, when we compare microbial communities from two soils with slightly different levels of resource availability. While a number of factors may concomitantly cause the differentiation of soil microbial communities, our data suggest that resource availability is the primary control on microbial community composition within the soil profiles.

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