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Soil morphology, depth and grapevine root frequency influence microbial communities in a Pinot noir vineyard

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[a]

Abstract

The composition of microbial communities responds to soil resource availability, and has been shown to vary with increasing depth in the soil profile. Soil microorganisms partly rely on root-derived carbon (C) for growth and activity. Roots in woody perennial systems like vineyards have a deeper vertical distribution than grasslands and annual agriculture. Thus, we hypothesized that vineyard soil microbial communities along a vertical soil profile would differ from those observed in grassland and annual agricultural systems. In a Pinot noir vineyard, soil pits were excavated to ca. 1.6–2.5 m, and microbial community composition in 'bulk' (i.e., no roots) and 'root' (i.e., roots present) soil was described by phospholipid ester-linked fatty acids (PLFA). Utilization of soil taxonomy aided in understanding relationships between soil microbial communities, soil resources and other physical and chemical characteristics. Soil microbial communities in the Ap horizon were similar to each other, but greater variation in microbial communities was observed among the lower horizons. Soil resources (i.e., total PLFA, or labile C, soil C and nitrogen, and exchangeable potassium) were enriched in the surface horizons and significantly explained the distribution of soil microbial communities with depth. Soil chemical properties represented the secondary gradient explaining the differentiation between microbial communities in the B-horizons from the C-horizons. Relative abundance of Gram-positive bacteria and actinomycetes did not vary with depth, but were enriched in 'root' vs. 'bulk' soils. Fungal biomarkers increased with increasing depth in 'root' soils, differing from previous studies in grasslands and annual agricultural systems. This was dependent on the deep distribution of roots in the vineyard soil profile, suggesting that the distinct pattern in PLFA biomarkers may have been strongly affected by C derived from the grapevine roots. Gram-negative bacteria did not increase in concert with fungal abundance, suggesting that acidic pHs in lower soil horizons may have discouraged their growth. These results emphasize the importance of considering soil morphology and associated soil characteristics when investigating effects of depth and roots on soil microorganisms, and suggest that vineyard management practices and deep grapevine root distribution combine to cultivate a unique microbial community in these soil profiles.
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Keywords: Vineyard; Grapevine; Soil resources; Carbon availability; Microbial community; Roots; PLFA; Fungi

1. Introduction

Gradients in soil resources, such as soil organic matter, soil nutrients, and moisture, are important drivers of soil microbial community composition (Bååth et al., 1995; Bossio and Scow, 1998). Roots are a major contributor to soil organic matter due to fine root turnover and rhizodeposition (Helal and Sauerbeck, 1989; Robinson and Scrimgeour, 1995; Shamoot et al., 1968). The vertical distribution of these root-derived organic carbon (C) inputs likely varies due to differences in rooting depth among plant species and communities (Gill et al., 1999; Jackson et al., 1996), but the extent of this variation is not

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well characterized. Soil microorganisms existing in horizons less strongly influenced by C inputs from litter (e.g., C horizons) rely partly on root-derived C for maintaining activity and growth (Pietikainen et al., 1999). Thus, it may be hypothesized that the vertical composition and distribution of soil microorganisms would be impacted by root distribution (Fierer et al., 2003; Potthoff et al., 2005). In annual grasslands, the quantity of soil organic matter has been correlated with the vertical distribution of soil microbial communities (Fierer et al., 2003). However, annual grasses have a considerably more shallow vertical root distribution than the dominant species in woody perennial ecosystems (Jackson et al., 1996) or a cultivated vineyard (Smart et al., 2006). As such, one might expect the distribution of soil microorganisms associated with grapevines in vineyards to differ from comparatively shallow-rooted grasslands and annual cropping systems, which could lead to distinctions in decomposition processes and nutrient turnover.

In addition to root-derived C inputs, soil management practices and soil chemical and physical characteristics such as moisture, texture, pH, and fertility influence microbial community properties like activity, biomass and composition (Bååth et al., 1995; Bardgett et al., 1997, 1999; Bossio et al., 1998; Lundquist et al., 1999). The effects of soil resource availability (i.e., soil C and moisture), soil physical and chemical characteristics, and abiotic conditions on soil microbial community composition have been studied in annual cropping systems, grasslands, wetlands, and forest systems (Bardgett et al., 1999; Fritze et al., 2000; Kelley and Hentzen, 2001; Pankhurst et al., 2002; Steenwerth et al., 2002). Comparatively less information exists on soil microbial community composition in woody perennial agroecosystems like vineyards, and previous investigations only examined such composition in the surface horizon (Drenovsky et al., 2005; Ingels et al., 2005). Vineyard microbial communities may be unique compared to other agroecosystems because vineyards have lower nitrogen fertilization inputs and experience relatively less-frequent tillage and fewer herbicide applications. These management practices have been linked to shifts in soil microbial communities in other ecosystems (Engelen et al., 1998; Calderón et al., 2000; Okano et al., 2004).

As soil microorganisms play a crucial role in ecosystem processes, it is important to understand influences of soil heterogeneity and grapevine root distribution on soil microbial communities in vineyards due to recent expansion of vineyard systems worldwide (USDA-NASS, 2004). In this study, we investigated how vertical root distribution and changes in soil resource availability and soil chemical and physical properties characteristics alter vineyard soil microbial communities with increasing depth in the soil profile. Due to deeper grapevine root distributions, we hypothesized that changes in microbial communities with increasing depth would differ from other ecosystems with more shallow rooted species. The vineyard used in this study exists on a heterogeneous site containing two soil types and a diverse array of slope characteristics. Therefore, we addressed the secondary hypothesis that soil heterogeneity may exert a greater influence over the soil microbial community composition than soil depth, per se.

2. Methods

2.1. Site description

Our investigations took place in August 2004 at a vineyard in the Carneros region of Napa County, CA (lat. 38° 14′ 49″ and long. 122° 21′ 59″). The climate is Mediterranean, with warm, dry summers and cool, wet winters. Mean annual precipitation for the region is 585 mm (10-year average; CIMIS), with small annual amplitudes in daily mean temperatures (14.1 °C in fall, 8.8 °C in winter, 12.3 °C in spring, 17.1 °C in summer; CIMIS). The slope on which the vineyard was established was modified to fill a pre-existing gully. The resulting topography is that of a series of concavities and convexities, with slope angle varying from 2 percent to 24 percent (Fig. 1). On the lower slopes of the vineyard, the soils are Haire Clay Loam series (fine, mixed, superactive, thermic Typic Haploxerult) while on the upper slopes the soils are Diablo Clay series (fine, montmorillonitic, superactive, thermic Typic Pelloxerert).

The vineyard covers ca. 6.25 ha. It is planted to Pinot noir UC 2A on 3309C rootstock, and the grapevines were planted in 1991. Vine rows are spaced 2.5 m apart and within row spacing is 1.5 m. The vines are trained as unilateral cordons with vertical shoot positioned (VSP) trellising and are drip irrigated. Annual grasses and forbs are allowed to grow in the middle region between the vine rows and are disced in the spring. Berms underneath the grapevines are kept clean with herbicide applications of glyphosate (RoundUp Ultramax™) and oryzalin (Goal2XL™) at recommended label rates.

Three sets of soil pits were excavated to a depth of ca. 1.5–2 m at three slope positions to capture the variation in topography and soil type, as determined by previously collected soil cores from 0 to 2 m (J.-J. Lambert, unpublished data). Three parallel transects were established on the slope, along which three pits per transect were excavated. To account for slope heterogeneity in the vineyard, three soil pits were on the shoulder of the slope, three were in the midslope, and three were in the toeslope. The pits had the dimensions of 0.6 m width by 2.0–2.5 m depth and 4 m in length at approximately 0.25 m away from the vine rows. In each pit, the top four horizons were sampled for soil microbial communities in soil around grapevine roots ('root') and in bulk soils ('bulk', i.e., without roots) and for soil characteristics in 'bulk' soil.

2.2. Soil morphology and analyses

The soil profile in each pit was classified according to the USDA method of soil taxonomy (Soil Survey Staff, 1997).

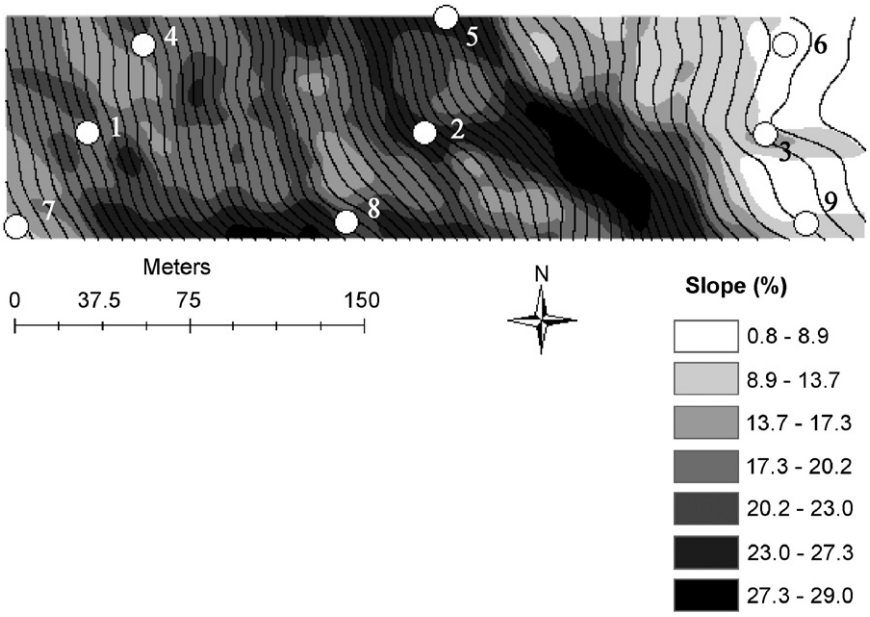

Fig. 1. Map of vineyard indicating percent slope, contours, and pit positions. Numbers next to circles indicate pit identity. Pits 1, 4, and 7 sit at the top. Pits 2, 5, and 8 are positioned at the midslope, and pits 3, 6, and 9 are on the toeslope.

Morphological designations were assigned to each horizon. Mean thickness ($n = 9$ pits) of depths 1–4 was 0–36, 36–70, 70–104 and 104–138 cm, respectively. Depth 1, the surface depth, represented the Ap horizon. Depth 2 corresponded to the next subtending horizon and included morphological designations of A, BAt, Bt1, AC, and C1. Depth 3 was positioned below depth 2 and included Bt1, Bt2, C1, C2, and 2C2. Depth 4, the lowest depth, included Bt2, BCt, and C2 horizons.

Bulk density of each horizon was measured using metal brass rings that were 6 cm deep with a volume of 332 cm^3. Soils dried at 105 °C for 48 h were used to determine gravimetric water content (GWC). Air dry soil samples were sieved through a 2 mm sieve. The <2 mm fraction was analyzed for exchangeable cations (x-K, x-Na, x-Ca, x-Mg; see Thomas, 1982), cation exchange capacity (CEC), pH by the saturated paste method (US Salinity Lab Staff, 1954), total C and nitrogen (N) by combustion (Pella, 1990), and particle-size distribution (i.e., sand, silt and clay) (Gee and Bauder, 1986). All soil analyses were conducted by Department of Agriculture and Natural Resources Analytical Lab (URL: danranlab.ucdavis.edu).

2.3. Root frequency measurements

Root depth distributions were determined using the point intercepts method on the soil profiles (Bohm, 1979). A grid with cell sizes of 20 cm width and 30 cm depth was established between the trunks of two adjacent vines. In-row vine spacing was 1.5 m, so 7 grids were established horizontally and depth ranged between 1.6 and 2.4 m depending on soil depth. Thus, from 5 to 8 horizontal rows were established in accordance with rooting depth. Root counts were expressed as a proportion and fitted to a model describing cumulative distribution with depth using the relation

$$Y = (1 - \beta^d), \quad (1)$$

where Y is the cumulative fraction and d the depth in the profile (Gale and Grigal, 1987; Smart et al., 2006).

2.4. Soil microbial community analyses

In each horizon, the 'root' and 'bulk' soils were sampled for soil microbial community analysis using a small diameter soil corer (2.5 cm). Three cores per horizon were collected in either 'root' or 'bulk' soil and then combined. In between each sample, the soil corer was cleaned with isopropyl alcohol and wiped dry. Soil samples were placed immediately on ice and stored in a −80 °C freezer until phospholipid ester-linked fatty acids (PLFA) extraction and analysis. The protocol for PLFA analysis followed Bossio and Scow (1998). Fatty acid nomenclature follows Steenwerth et al. (2002). PLFA were expressed as nmol PLFA g^{-1} dry soil.

Thirty-eight individual PLFA were included in the statistical analysis. PLFA were grouped into bacterial, fungal, protozoan and unknown identity following Kroppenstedt (1985), Federle (1986), O'Leary and Wilkinson (1988), Vestal and White (1989), Olsson et al. (1997), Zelles (1997) and Bossio and Scow (1998). A detailed grouping is given in Table 3. The sum of the 38 fatty acids served as a measure of total microbial biomass, or Total PLFA (Zelles, 1997). Ratios of cy19:precursor (18:1ω7c) and saturated:monoenoic PLFA were calculated to as indices of nutritional stress (see Kieft et al., 1997; Bossio and Scow, 1998).

2.5. Statistical analysis

The relationships between soil microbial communities and Pit (9 locations), Depth (4 depths per pit), and Root (root presence or absence) were analyzed by canonical correspondence analysis (CCA) using the nominal variables of Pit, Depth and Root as environmental variables (Canoco, version 4.5, Microcomputer Power, Inc., Ithaca, NY; ter Braak, 1987). All environmental variables were tested for significant contribution ($P<0.05$) to the explanation of the variation in the PLFA data using the Monte Carlo permutation.

The relationship among soil microbial community composition and soil characteristics was analyzed by a second CCA. Due to the small amount of recoverable soil from root samples, soil characteristics were determined only for 'bulk' soil samples. As such, only PLFA samples from 'bulk' soil were included in the analysis. The significance of the soil characteristics in the CCA was determined using the Monte Carlo permutation test.

Soil microbial groups (i.e., actinomycetes, fungi, protozoa, Gram-positive, Gram-negative and unknown markers) and Total PLFA were analyzed by analysis of variance (ANOVA) using a split plot design with Kenward–Roger as the degrees of freedom method. Depth was a main plot factor, and Pit was a sub-plot effect (PROC MIXED, SAS version 8.01, Cary, NC). Pit location was treated as a random factor. Mean separation tests for Root and Depth were conducted using least squared means for the Bonferroni post hoc test ($P<0.05$). The relationship between microbial groups and root frequency was determined by multiple linear regression (PROC REG, SAS version 8.01, Cary, NC; $P<0.05$) and Pearson's correlation coefficient. Weighted averages were used to adjust the depths on the root-sampling grid to match those of the soil morphological horizons. Due to limited degrees of freedom, bulk soil characteristics were analyzed by ANOVA (PROC GLM, SAS version 8.01, Cary, NC; $P<0.05$) and only tested for the effect of Depth. Mean separation tests for Depth were conducted using least-squared means for the Bonferroni post hoc test.

3. Results

3.1. Soil characteristics and root distribution

The range in the variation of soil characteristics among pits and the associated depths tended to be high (Table 1). Bulk density (Db) in the surface depth was less than in the three lower depths. Gravimetric water content (GWC) did not differ among depths, but values within a given depth varied 2–3 fold. A similar trend was observed for CEC and x-Ca. Exchangeable K, commonly applied as potassium nitrate during fertigation, was 1.25 times greater in the surface than in the lower depths. In contrast, x-Mg and x-Na increased 1.5 and 7.5 times, respectively, from the surface to lowest depths. Sand, silt and clay content displayed a wide range in values, but did not change with increasing depth. Soil pH tended to vary between slightly acidic to neutral (pH 5.5–7.2) in the upper two depths and from acidic to slightly basic (pH 5.2–8.3) in the lower depths. Total C decreased by half between the surface and second uppermost depth, and again between the second depth and the two lowest depths. However, percent CV's for Total C indicated a wide range in values within a given depth among all pits. Trends for Total N were similar to Total C. Therefore, among the nine soil pits, soil heterogeneity was relatively high.

Root frequency tended to decrease with increasing depth (Fig. 2). The upper two depths had similar root frequencies (depth 1: 32.0 ± 2.9, depth 2: 37.6 ± 4.1, $n=9$), but from the second upper depth to the immediate subtending depth (depth 3), root frequency values decreased by ca. 55% ($P<0.05$; depth 3: 20.0 ± 1.6, $n=9$) and then again by ca. 50% between the third and fourth depths ($P<0.05$; depth 4: 10.4 ± 2.5, $n=9$). In individual soil pits, the root frequency as a percentage of total root intercepts ranged from 5% to 40% in depth 1, 20–46% in depth 2, 17–39% in depth 3, and 2–19% in depth 4 (see Fig. 2). Using the relationship $Y=(1-\beta^d)$, where Y is the cumulative root distribution as a fraction of 1, d the depth (cm), and β is an estimated coefficient, the relative depth distribution of roots among pits can be compared. Greater values in β indicate that roots were more deeply distributed (Gale and Grigal, 1987). When analyzing the root distribution from the surface to depths ranging between 1.6 and 2.4 m, β values ranged between 0.97 and 0.98, indicating that the depth distribution of roots was relatively deep in these soil pits compared to temperate grasslands and tundra ecosystems (Jackson et al., 1996).

3.2. Canonical correspondence analysis of PLFA

Microbial communities clustered according to depth when PLFA from both 'root' and 'bulk' soils were analyzed together (data not shown). In the CCA biplot of PLFA that were constrained with Pit, Depth and Root, Axis 1 explained 24.6% of the variation, and Axis 2 explained 39.1% of the variation. Depth was highly correlated with Axis 1, and thus accounted for a large amount of the variation in the distribution of samples along that axis. Microbial communities from the surface Ap horizon (i.e., Depth 1) clustered together to the left along Axis 1. The Bt1 and Bt2 horizons (i.e., Depths 2–4) tended to form a diffuse cluster to the right along Axis 1. BC, C1 and C2 horizons (i.e., Depths 3 and 4) tended to be located on the positive sides of Axes 1 and 2 (i.e., the upper right quadrant). Vectors for Pit and Root were not highly correlated with either axis, as indicated by their non-significant loading with respect to either axis.

In general, surface soils in the Ap horizon were more enriched in PLFA (i.e., ca. 68% of PLFA with loading scores $>|\pm1|$ had negative scores for Axis 1; see Table 2). PLFA biomarkers that were enriched in the Ap horizons

Table 1
Means of soil characteristics ($n = 9$)

Soil characteristics	Depth 1		Depth 2		Depth 3		Depth 4	
	Mean ± SE	CV (%)	Mean ± SE	CV (%)	Mean ± SE	CV (%)	Mean ± SE	CV (%)
Total C ($g\,kg^{-1}$)	10.32 ± 0.83^{a}	24.1	5.2 ± 1.1^{b}	63.7	2.46 ± 0.48^{c}	58.2	2.47 ± 0.60^{c}	73.0
Total N ($g\,kg^{-1}$)	$1.10 \pm .06^{a}$	17.0	0.70 ± 0.08^{b}	34.1	0.48 ± 0.04^{c}	25.2	0.42 ± 0.02^{c}	15.7
Sand (%)	45.0 ± 4.1^{a}	27.5	48.0 ± 6.2^{a}	38.6	48.1 ± 8.6^{a}	54.0	45.3 ± 8.5^{a}	56.4
Silt (%)	25.8 ± 2.3^{a}	24.7	22.8 ± 3.1^{a}	41.0	20.0 ± 3.8^{a}	56.5	23.1 ± 4.1^{a}	53.0
Clay (%)	29.2 ± 2.9^{a}	29.8	29.2 ± 4.0^{a}	41.3	31.9 ± 5.1^{a}	48.4	31.6 ± 5.2^{a}	49.6
pH	6.4 ± 0.2^{a}	8.6	6.7 ± 0.3^{a}	13.1	7.0 ± 0.4^{a}	15.5	7.2 ± 0.4^{a}	17.6
CEC* ($cmol\,kg^{-1}$)	28.3 ± 2.6^{a}	27.6	26.7 ± 3.3^{a}	37.0	29.0 ± 4.2^{a}	42.9	30.1 ± 3.7^{a}	36.8
x-K ($cmol\,kg^{-1}$)	0.4 ± 0.0^{a}	30.2	0.3 ± 0.0^{b}	23.3	0.3 ± 0.0^{b}	37.0	0.3 ± 0.0^{b}	33.8
x-Ca ($cmol\,kg^{-1}$)	12.8 ± 1.3^{a}	29.9	12.3 ± 1.4^{a}	34.4	13.0 ± 1.5^{a}	33.7	16.1 ± 2.5^{a}	46.3
x-Mg ($cmol\,kg^{-1}$)	6.6 ± 1.2^{a}	53.9	7.6 ± 1.6^{ab}	61.9	9.5 ± 2.0^{ab}	63.5	10.2 ± 2.0^{b}	59.7
x-Na ($cmol\,kg^{-1}$)	0.2 ± 0.0^{a}	42.0	0.4 ± 0.1^{ab}	92.9	1.0 ± 0.3^{bc}	90.8	1.5 ± 0.5^{c}	96.8
Soil Moisture (g/g)	12.23 ± 1.08^{a}	26.6	13.74 ± 1.45^{a}	31.6	15.50 ± 2.44^{a}	47.3	13.2 ± 1.2^{a}	25.2
Bulk Density (g/cm^3)	1.24 ± 0.06^{a}	14.6	1.38 ± 0.05^{b}	10.1	1.47 ± 0.07^{b}	13.3	1.44 ± 0.06^{b}	12.6

Superscript letters indicate significant differences by ANOVA and Bonferroni mean separation test ($P < 0.05$).
*'CEC' is 'cation exchange capacity'.

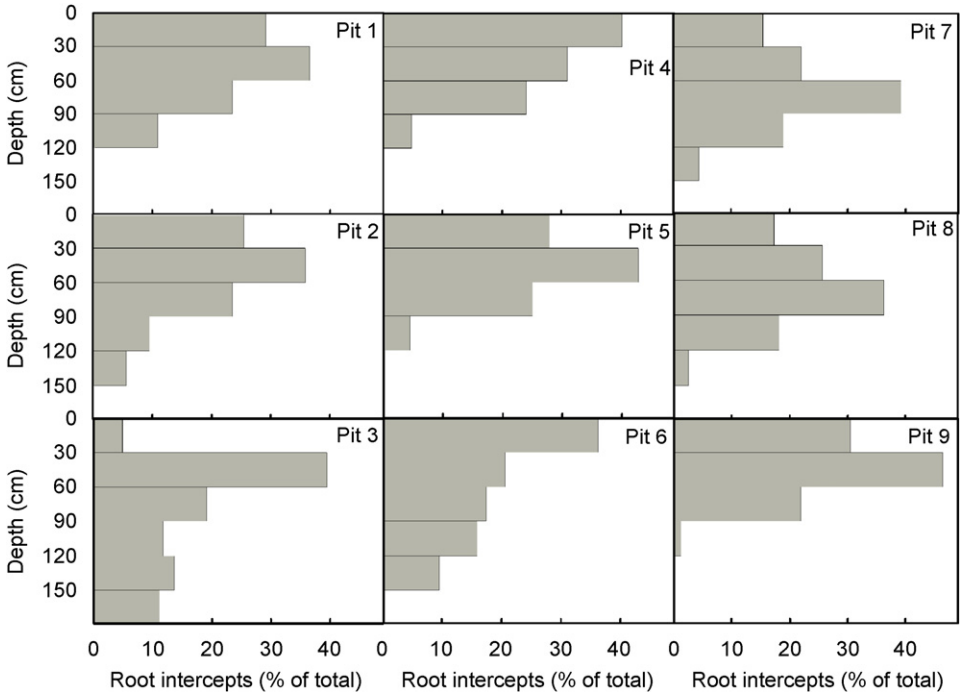

Fig. 2. Root frequency distribution of each soil pit.

on Axis 1 were 17:1ω9c (i.e., Gram-negative; O'Leary and Wilkinson, 1988), 17:0 10Me (i.e., actinomycetes; Kroppenstedt, 1985), and 16:1ω5c (i.e., Gram-negative, arbuscular mycorrhizae; Nordby et al., 1981; Olsson and Johansen, 2000). Other PLFAs were enriched in the Ap horizon but were not linked to specific microbial groups. PLFA biomarkers that were associated with lower depths were 18:2ω9,6c (i.e., fungi; Federle, 1986) and 18:1ω9c (i.e., Gram-negative, fungi, and microeukaryotes; Nordby et al., 1981; Findlay and Dobbs, 1993; Kourtev et al., 2003).

When the CCA of soil microbial community samples from 'bulk' soil was constrained with associated soil characteristics, Axes 1 and 2 explained 23.9% and 8.8% of the variation, respectively (Fig. 3a). Microbial communities in the Ap horizon clustered tightly to the left along Axis 1, and communities from lower depths were located to the right along Axis 2 in a similar arrangement as the CCA constrained by Pit, Depth and Root. Axis 2 separated microbial communities of the A and B horizons from the C horizon.

Soil characteristics that were significant in explaining the variation in PLFA were correlated with the first axis and were associated with microbial communities in the Ap horizon (Monte Carlo, $P < 0.05$; Fig. 3b). These

Table 2
Biplot scores $>|\pm 1|$ of PLFA from CCA (See Fig. 3)

PLFA	CCA of PLFA in bulk and root soil (CCA not shown)		CCA of PLFA in bulk soil (Fig. 3)	
	Axis 1	Axis 2	Axis 1	Axis 2
17:1ω9c	−2.866	n.a.	−3.090	1.640
15:1 iso f	−2.726	n.a.	−2.683	2.049
20:0	−2.247	n.a.	−2.478	2.675
17:0 10Me	−1.745	n.a.	−1.843	n.a.
16:1 ω5c	−1.673	n.a.	n.a.	1.096
18:1 iso h	−1.612	n.a.	−3.540	n.a.
16:1ω11c	−1.554	−1.067	n.a.	n.a.
iso17:1ω5c	−1.348	n.a.	n.a.	−1.220
14:0iso	−1.256	n.a.	n.a.	n.a.
14:0	−1.126	n.a.	−1.9205	1.235
17:0	−1.010	n.a.	n.a.	n.a.
16:1 2OH	1.132	−1.101	−1.729	n.a.
Sum6	3.035	3.516	−2.117	1.554
18:1 ω9c	n.a.	1.144	1.345	1.043
19:0cy	n.a.	n.a.	−1.638	n.a.
16:0	n.a.	n.a.	1.223	1.227
16:1ω7c	n.a.	n.a.	n.a.	−3.995
15:0 3OH	n.a.	n.a.	n.a.	−1.536
17:0 anteiso	n.a.	n.a.	n.a.	−1.289

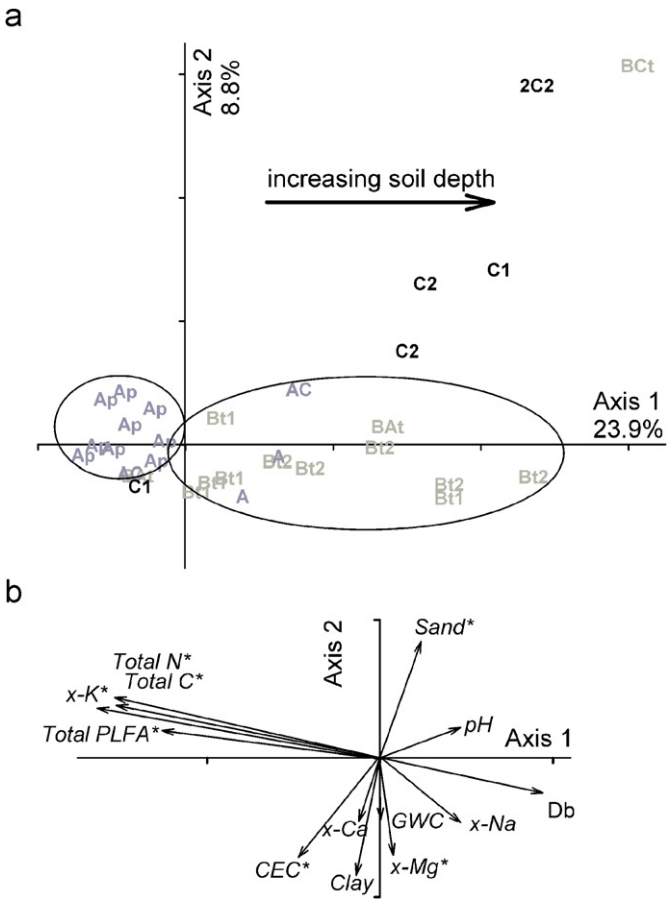

Fig. 3a and 3b. Canonical correspondence analysis of soil microbial communities from 'bulk' soil constrained soil chemical and physical characteristics. The depiction of the soil microbial community (Fig. 3a) is separated from soil chemical and physical characteristics (Fig. 3b) for clarity. Asterisks indicate that the variable is significant by the Monte Carlo permutation test ($P<0.05$). Symbols (e.g., Ap, Bt1, Bt2, etc.) indicate soil morphological horizons as described in Section 2. Polygons are used to clarify groupings.

environmental variables were Total N, Total C, Total PLFA (i.e., labile C), and x-K, indicating that the surface horizon had higher concentrations in these soil resources (also, see Table 1). Bulk Density (Db) and pH were correlated with Axis 1 in the positive direction, where associations with lower horizons corresponded to higher bulk density and a wide range in soil pH.

Soil characteristics that were associated with the second axis in the negative direction and positively correlated with each other included x-Ca, gravimetric water content (GWC), x-Mg, CEC, and Clay (Fig. 3b). Exchangeable cations and CEC were associated with clay mineralogy and clay accumulation in Bt1 and Bt2 horizons. Sand was positively associated with Axis 2, indicating that the C horizons tended to contain relatively more sand than the B horizons.

As in the first CCA, PLFA tended to be enriched in the surface depths (i.e., ca. 63% of PLFA with loading scores $>|\pm 1|$ had negative scores for Axis 1; Table 2). Similar biomarkers also tended to be enriched in the same respective horizons.

3.3. PLFA biomarker groups

Approximately 30–80% of the total PLFA biomass resided in the surface layer. Total PLFA (nmol g^{-1}) decreased with increasing depth and was ca. 2.5–6 times greater in the upper (i.e., the Ap horizon) than lower depths ($P<0.0001$, $F=32.54$; Table 3). The biomass of each individual biomarker group also decreased significantly with depth ($P<0.0001$), with the exception of protozoan biomarkers, which were present only in the surface. Decreases in biomass between the second and third depths only occurred with actinomycetes, which decreased by ca. 45% between these layers. Decreases in any biomarker between the lower two depths did not occur.

Biomass of PLFA biomarkers did not respond consistently between 'root' and 'bulk' soils. Total PLFA was ca. 60% greater in samples with roots than bulk soil across all depths and pits ($P=0.0229$, $F=5.76$; 'root': 13.22 ± 1.96 nmol PLFA g^{-1}, 'bulk': 8.76 ± 1.69 nmol PLFA g^{-1}). Fungal PLFA was the only biomarker to have greater biomass in 'root' than 'bulk' soils ($P<0.0001$, $F=17.88$; 'root': 2.42 nmol PLFA g^{-1}, 'bulk': 1.10 nmol PLFA g^{-1}). Unknown PLFA markers also had ca. 1.5 times more biomass in 'root' than 'bulk' soil ($P=0.0204$, $F=5.99$). In contrast, Gram-positive biomarkers were ca. 20% greater in 'bulk' than 'root' soil ($P=0.0419$, $F=4.38$), and actinomycetes displayed the same trend ($P=0.0504$, $F=4.23$). Protozoa and Gram-negative bacteria did not differ between 'root' and 'bulk' soils.

Table 3
Means of microbial biomarkers and stress markers

Biomarkers (nmol g^{-1})	Depth 1	Depth 2	Depth 3	Depth 4
Gram-negative[1]	3.96 ± 0.42^{a}	1.50 ± 0.31^{b}	0.96 ± 0.25^{bc}	0.40 ± 0.08^{c}
Gram-positive[2]	6.38 ± 0.80^{a}	2.96 ± 0.64^{b}	1.71 ± 0.45^{bc}	0.83 ± 0.16^{c}
Actinomycetes[3]	1.80 ± 0.18^{a}	0.91 ± 0.20^{b}	0.55 ± 0.15^{c}	0.19 ± 0.04^{c}
Fungi[4]	3.44 ± 0.42^{a}	0.98 ± 0.18^{b}	1.10 ± 0.27^{b}	1.08 ± 0.40^{b}
Protozoa[5]	0.07 ± 0.02^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
Non-specific[6]	6.96 ± 0.80^{a}	2.80 ± 0.57^{b}	1.90 ± 0.46^{b}	1.01 ± 0.24^{bc}
Total PLFA	22.61 ± 2.60^{a}	9.14 ± 1.86^{b}	6.67 ± 1.52^{bc}	4.01 ± 0.82^{c}
Ratios				
cy19:pre	0.10 ± 0.01^{a}	0.09 ± 0.03^{a}	0.13 ± 0.05^{a}	0.12 ± 0.06^{a}
Saturated: monoenoic	1.00 ± 0.01^{a}	1.34 ± 0.06^{b}	1.27 ± 0.05^{b}	1.91 ± 0.14^{c}

Superscript letters indicate significant differences by ANOVA and Bonferroni Mean Separation test ($P < 0.05$).

[1]16:1ω7c, 16:1ω5c, 15:0 2OH, 15:0 3OH, 17:1ω9c, 17:0cy, 16:1 2OH, 19:0cy (Federle, 1986; O'Leary and Wilkinson, 1988; Vestal and White, 1989).

[2]13:0i, 13:0a, 14:0i, 15:0i, 15:0a, 16:0i, 16:0a, 17:0i, 17:0a (O'Leary and Wilkinson, 1988).

[3]16:0 10Me, 17:0 10Me, 18:0 10Me (Kroppenstedt, 1985; O'Leary and Wilkinson, 1988; Vestal and White, 1989).

[4]Sum of 18:3ω6,9,12c, 18:1ω9c, 18:2ω6,9c, sum7 (i.e., an unresolved mixture of 18:1ω7c, 18:1ω9t, 18:1ω12t, and 18:1ω9c) (Federle, 1986; O'Leary and Wilkinson, 1988; Vestal and White, 1989; Frostegård et al., 1993; Zelles, 1997).

[5]20:4 ω 6,9,12,15c, 20:2ω6,9c (White et al., 1996).

[6]14:0, 15:1i f, 15:0, 16:1ω7t, 16:1ω11c, 16:0, unk 17:1i, 17:1ω5c, 17:0, 18:1i, 18:0, 20:0 and sum9 (i.e., an unresolved mixture of 19:0cy ω10c and two unknown PLFA).

The relative abundance of biomarkers (i.e., percent of total biomass) was compared within and across soil depths to understand the relative changes in dominance by each group with increasing depth (data not shown). The relative abundance of Gram-positive bacteria including actinomycetes and the remaining unknown PLFA did not change with increasing depth. Protozoa and Gram-negative bacteria decreased in relative abundance only between the surface and second depth [ca. 20–25% decrease in Gram-negative ($P = 0.0171$, $F = 4.31$), and protozoa present only in surface ($P = 0.0001$, $F = 8.36$)]. Fungal PLFA was the only biomarker group to display distinct responses in 'root' and 'bulk' soils with depth (Table 3; depth × root, $P = 0.0034$, $F = 5.82$). In 'root' soils, relative abundance of fungal markers in the lower two depths was twice as great as in the upper two depths ('root' soils: Depth 1, $16.4 \pm 1.3\%$; Depth 2, $16.8 \pm 3.0\%$; Depth 3, $30.3 \pm 4.6\%$; Depth 4, $33.8 \pm 6.2\%$), while their relative abundance in 'bulk' soils did not change with depth ('bulk' soils: Depth 1, $13.6 \pm 1.2\%$; Depth 2, $15.0 \pm 5.4\%$; Depth 3, $13.7 \pm 4.1\%$; Depth 4, $9.2 \pm 2.5\%$). Likewise, the ratio of fungi:bacteria markers increased from 0.21 in the two upper depths to 0.47 and 0.59 in the third and fourth depths, respectively, in 'root' soil only ($P = 0.0059$, $F = 9.74$; data not shown).

Table 4
Multiple regressions of root frequency on microbial biomarkers and Pearson correlation coefficients

Biomarkers from 'Root' soil	Pearson correlation coefficient	Linear regression of relative proportions of biomarker groups on root frequency		
		P	F	R^2
Gram-negative	0.507	0.0042	9.72	0.2576
Gram-positive	0.404	0.0270	5.45	0.1630
Actinomycetes	0.550	0.0017	12.12	0.3020
Fungi	−0.523	0.0030	10.54	0.2734
protozoa	0.103	0.5871	0.30	0.0107
Unknown bacteria	0.234	0.2135	1.62	0.0547
Total PLFA	0.254	0.1758	1.93	0.0645

Linear regression of the proportion of specific biomarker groups from 'root' soil on root frequency was significant for Gram-negative, Gram-positive, and actinomycetes (Table 4). Pearson's correlation coefficients revealed a positive relationship between these biomarkers and root frequency, while fungal biomarkers exhibited a negative relationship.

The ratio of saturated:monoenoic PLFA increased 30% between the surface and the next two depth intervals, and then again by ca. 45% between the third and lowest depth ($P < 0.0001$; $F = 33.36$) (Table 3). The ratio of cy19:precursor did not change with depth in 'bulk' soils, but in 'root' soils the ratio remained constant in the upper three depths and then doubled between the third depth and the lowest depth (depth 3: 0.10 ± 0.06 nmol g^{-1} vs. depth 4: 0.24 ± 0.01 nmol g^{-1}; depth × root: $P = 0.0084$, $F = 4.93$).

4. Discussion

4.1. Shifts in soil microbial communities with soil resource gradients

We hypothesized that changes in soil resource availability at depth would drive soil microbial community composition, but that within-site variation in other soil morphological factors could outweigh the potential effects of any singular or set of soil resources on microbial community composition. In fact, there were clear differences in soil characteristics and resources at different depths, and in particular, among specific soil morphological horizons. Soil C and N, and Total PLFA, a measure of labile C, were most strongly associated with soil microbial communities from the Ap horizon, indicating that these soil resources played a role in differentiating surface communities from those at lower depths. In California's annual grasslands, decreased soil C and moisture were associated with shifts in microbial communities and increases in the ratio of saturated:monoenoic PLFA (Fierer et al., 2003; Potthoff et al., 2005), an indicator of increasing

nutritional stress in microorganisms (Kieft et al., 1994). Here, the ratio increased with increasing depth and paralleled the decrease in total C and labile C (i.e., total PLFA), suggesting resource limitation at lower soil depths.

Other soil chemical and physical characteristics tended to play a secondary role in the clustering of soil microbial communities. Factors known to affect soil microbial community composition such as soil texture (i.e., sand and clay), bulk density, exchangeable cations, CEC, and soil moisture were more important in explaining the differences between the B and C horizons. These soil physical factors (e.g., soil texture and bulk density) affect the soil microclimate experienced by soil microbes, including temperature fluctuations, gas concentrations and exchange, and soil water potential and holding capacity (Hillel, 1982). Therefore, differences in microclimate related to these factors may have influenced soil microbial community composition in the vineyard to some extent (Zogg et al., 1997; Bossio and Scow, 1998).

Nonetheless, we suggest that soil C played a more important role in the differentiation of soil microbial community composition than soil moisture or these soil physical factors. Soil moisture content is often classified as a soil resource for microbes. It was relatively greater in B horizons, but its vector, and thus its explanatory power, was diminished relative to other soil chemical characteristics associated with soil microbial communities in subsurface horizons. Also, no taxonomic features in the profile indicated that anaerobic conditions had occurred in the soil profile (J-.J. Lambert, data not shown), which also supports our supposition that steep soil moisture gradients were not dominant features of these soil profiles.

Furthermore, soil moisture alone is less likely to have an impact on community composition than is organic C addition in some agricultural soils (Drenovsky et al., 2004). When Drenovsky et al. (2004) adjusted soil moisture of agricultural soils to air dry, half field capacity, field capacity, and flooded conditions, soil microbial community composition displayed only slight shifts with changes in soil moisture. Upon the addition of organic C, the magnitude of change displayed by the microbial community composition was much greater at all soil moisture levels than that exhibited by flooded soils without add C. These observations suggest that soil C, and not soil moisture, was a main factor behind the change in microbial community composition.

It appears in our study that two gradients, one comprised mainly of soil resources (i.e., soil C and N, x-K, and labile C) and the other of soil chemical and physical characteristics, influenced the distribution of microbial communities in the soil profile (see Fig. 3b). In managed and undisturbed ecosystems such as temperate forests, agricultural systems, and native and exotic grasslands, soil microbial communities also have been observed to differ based on distinctions in soil resources and soil physical factors (Bardgett et al., 1999; Myers et al., 2001; Steenwerth et al., 2002).

4.2. Microbial community composition as a function of depth

The grapevine root distribution in this study was relatively deep, similar to what has been previously reported for grapevines in other vineyards and for early successional tree species, desert shrubs, and conifers. This is in contrast to the more shallow root distribution observed in temperate grasslands and tundra ecosystems (Gale and Grigal, 1987; Jackson et al., 1996; Smart et al., 2006) and suggests that woody species like grapevine may support increased rhizodeposition at depth. In this context, we posed our hypothesis that with increasing depth, vineyard soil microbial communities would differ from those in ecosystems with shallower rooting depths and potentially resemble microbial communities in other systems with deeper root distributions.

The distribution of specific microbial populations in vineyard soils was not consistent with previous studies in grasslands and cotton fields. Previously, in Mediterranean grasslands in California, Gram-positive biomarkers, including actinomycetes, increased with depth (Fierer et al., 2003; Potthoff et al., 2005). This shift was attributed to the ability of these organisms to mineralize recalcitrant organic compounds under low oxygen concentrations or anaerobic conditions (Goodfellow and Williams, 1983). Furthermore, the proportion of Gram-negative and fungal biomarkers correspondingly decreased, which was attributed to their zymogenic nature and their dependence on inputs of fresh organic material (Griffiths et al., 1999). These surveys were conducted along soil profiles that were one (Potthoff et al., 2005) and two (Fierer et al., 2003) meters in depth, corresponding to the range of depths collected in the current study., Similar trends were observed in a long-term cotton plantation, although the sampling depth was shallower (0–24 cm) (Feng et al., 2003). In contrast, there was no change in the proportion of Gram-positive bacteria and actinomycetes with depth in the current study. While the proportion of Gram-negative bacteria did decrease with depth, the proportion of fungal biomarkers actually increased with depth, but only in soil sampled around roots. It is uncertain why the relative abundance of Gram-negative bacteria did not increase in parallel with that of fungi. However, the more acidic pHs in some of the lower soil horizons may have selected preferentially for fungi, given their reduced sensitivity to acidic environments (Pennanen et al., 1998).

Why are microbial communities so different in vineyard soils? In our study, significant correlations between biomarker groups and root frequency as well as significant linear regression of specific biomarkers on root frequency imply a close relationship between the soil microbial communities and vertical root distribution. The ratio of cy19:precursor, an indicator of nutritional stress in bacteria, only increased in root soil of the deepest soil horizon in which root frequency was lowest (see Fig. 2 and Table 3), suggesting decreased root presence (and thus

lower labile C sources) may increase stress (Guckert et al., 1986; Kieft et al., 1997). Gram-positive bacteria, overall, did not vary with depth, despite their general acceptance as highly stress tolerant organisms. However, relative abundance of Gram-positive bacteria including actinomycetes was greater in 'bulk' (i.e., non-root) soils, where lower C availability would be expected to encourage dominance of these more stress-tolerant groups.

Additionally, the relatively deep root distribution in this vineyard compared to other systems may further explain the distinct vertical biomarker distribution. In more shallowly rooted systems, such as grasslands and annual agricultural systems, fungal populations generally are enriched in surface soils (Pankhurst et al., 2002; Feng et al., 2003; Fierer et al., 2003). We found contrary results in our deeply rooted vineyard soils. Although total fungal biomass was greater in surface soils, its relative abundance compared to that of other microbial groups was lowest in surface soils and greatest in deeper, undisturbed soils near roots. These data suggest grapevine rhizodeposition, in combination with shifts in soil characteristics, strongly influenced root-associated fungal populations in the soil profile. These deep roots likely supplied labile C to fungi through fine root turnover and root exudation. Root C has been documented as great as 20 mm away from the root, which is well within the sampling radius used in this study (Helal and Sauerbeck, 1989). Although forest systems typically follow patterns of fungal abundance observed in grasslands and agricultural systems (Fritze et al., 2000, 2003; Feng et al., 2003; Potthoff et al., 2005), associated understory plants likely contribute to comparative differences in soil microbial community composition, despite rhizodeposition from deeply rooted forest trees. Further differentiating our vineyard system, tillage, perhaps in combination with relatively low summer soil moisture, both of which are known to decrease fungal abundance, may have negative long-term effects on fungal communities (Frey et al., 1999; Guggenberger et al., 1999).

In partial support of the secondary hypothesis that soil heterogeneity across the vineyard would be a main driver of soil microbial community composition, soil morphological designation, especially in the lower depths, distinguished the soil microbial community composition, not pit location or root presence (data not shown). This observation is consistent with other studies that suggest that soil characteristics are important determinants of soil microbial communities (Bossio et al., 1998; Marschner et al., 2004). Support for such segregation also has been reported for podzol profiles under a coniferous forest, where microbial communities segregated by soil morphology (Fritze et al., 2000).

Nonetheless, in this investigation, soil depth was an important and distinguishing factor in separating microbial communities in the surface from the lower depths. The surface Ap horizon (depth 1) communities were most similar in composition, despite the extreme differences in whole pedon taxonomy between sampling pits. While these soils currently are not tilled in the sampling zone, they were tilled and graded when the vineyard was installed 13 years prior to sampling. Tillage practices can impact microbial community composition in both the short (i.e., days to months) and long-term (i.e., years), with differences evident even 7 years after tillage ceased in annual agroecosystems (Petersen and Klug, 1994; Calderón et al., 2000; Buckley and Schmidt, 2001; Feng et al., 2003; Jackson et al., 2003). Thus, the similarity in microbial communities across pedons in the surface soil of this vineyard may partly reflect the fact that these layers commonly shared the initial soil disturbance by tillage that occurred when the vineyard was planted. Extreme temperature changes and repeated wet–dry cycles, common to Mediterranean climates and experienced more strongly by the surface than deeper layers, also alter soil microbial communities and likely are two additional factors that shaped the emergence of distinctly different soil microbial communities in the surface compared to the lower layers (Zogg et al., 1997; Lundquist et al., 1999; Schimel et al., 1999).

Utilization of soil taxonomy in our study (i.e., morphological horizon designations) helped elucidate relationships between soil microbial communities, soil resources and other physical and chemical characteristics. These relationships were not evident when inspecting changes with root presence or depth alone because of substantial variation in soil morphological designations within a particular depth. Thus, these results emphasize the importance of considering whole-pedon soil morphology and its impact on soil conditions (e.g., soil pH) that influence microbial communities, as well as indicate that roots and microorganisms can interact differently depending on soil morphology. Moreover, soil resource gradients, followed by soil chemical characteristics, influenced soil microbial community distribution. Compared to other systems, the distinct patterns in soil microbial communities as influenced by depth and root distribution in this Pinot noir vineyard suggest that vineyard management practices and deep grapevine root distribution combine to cultivate a unique microbial community in these soil profiles. These shifts in functional groups of soil microorganisms raise the hypothesis that nutrient turnover and decomposition may be unique in these soils.

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