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Shifting microbial community structure across a marine terrace grassland chronosequence, Santa Cruz, California

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ABSTRACT

Changes in the biomass and structure of soil microbial communities have the potential to impact ecosystems via interactions with plants and weathering minerals. Previous studies of forested long-term (1000s – 100,000s of years) chronosequences suggest that surface microbial communities change with soil age. However, significant gaps remain in our understanding of long-term soil microbial community dynamics, especially for non-forested ecosystems and in subsurface soil horizons. We investigated soil chemistry, aboveground plant productivity, and soil microbial communities across a grassland chronosequence (65,000–226,000 yrs old) located near Santa Cruz, CA. Aboveground net primary productivity (ANPP) initially increased to a maximum and then decreased for the older soils. We used polar lipid fatty acids (PLFA) to investigate microbial communities including both surface (<0.1 m) and subsurface (≥0.2 m) soil horizons. PLFAs characteristic of Gram-positive bacteria and actinobacteria increased as a fraction of the microbial community with depth while the fungal fraction decreased relative to the surface. Differences among microbial communities from each chronosequence soil were found primarily in the subsurface where older subsurface soils had smaller microbial community biomass, a higher proportion of fungi, and a different community structure than the younger subsurface soil. Subsurface microbial community shifts in biomass and community structure correlated with, and were likely driven by, decreasing soil P availability and Ca concentrations, respectively. Trends in soil chemistry as a function of soil age led to the separation of the biological (≤1 m depth) and geochemical (>1 m) cycles in the old, slowly eroding landscape we investigated, indicating that this separation, commonly observed in tropical and subtropical ecosystems, can also occur in temperate climates. This study is the first to investigate subsurface microbial communities in a long-term chronosequence. Our results highlight connections between soil chemistry and both the aboveground and belowground parts of an ecosystem.

1. Introduction

Soil microbial communities are dynamic components of ecosystems, playing a major role in soil organic matter (SOM) decomposition as well as contributing to plant water and nutrient acquisition and mineral weathering. The size and structure of microbial communities affect soil nutrient cycling. Microbial communities are therefore key to understanding ecosystem-level processes including plant productivity (van der Heijden et al., 2008) and sensitivity to perturbation (Balser and Firestone, 2005).

Previous work found that soil microbial communities shifted along nutrient availability gradients such as agricultural versus undisturbed (Grayston et al., 2004; Potthoff et al., 2006), short-term chronosequences (<100 years) of agricultural versus restored grasslands (Allison et al., 2005, 2007b), or grasslands versus forests (Balser and Firestone, 2005; Waldrop and Firestone, 2006). The shift often included an increase in fungi with decreasing disturbance or nutrient availability. Studies that focused on microbial communities in subsurface (≥0.2 m) soils determined that microbial biomass decreased quickly and community structure varied with depth below the surface (Blume et al., 2002; Fierer et al., 2003; Potthoff et al., 2006; Allison et al., 2007b). Low C inputs (Richter and Markewitz, 1995) and slower turnover of C present in subsurface soils (Trumbore, 2000) were proposed to contribute to the decrease (Fierer and Jackson, 2006).

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Unlike soil C and N, primarily added by biological fixation, P is derived from soil minerals (Walker and Syers, 1976; Crews et al., 1995; Vitousek et al., 1997). With increasing soil age across long-term chronosequences, primary minerals containing P (e.g., apatite) are depleted by weathering, resulting in increased cycling of P through plant biomass and SOM with much of the P eventually leaving the soil system (Crews et al., 1995; Vitousek et al., 1997). In addition, common pedogenic minerals such as iron (Fe) oxides sorb and co-precipitate with P, making P less available in highly weathered soils with abundant Fe oxides (Walker and Syers, 1976). Due in part to these pedogenic process, forest ecosystems on long-term chronosequences (1000s to 1000,000s of years) typically increase in productivity from the youngest soils to middle-aged soils, and exhibit declining productivity in the oldest soils (e.g., Vitousek et al., 1997; Wardle et al., 2004b). Productivity changes are driven by N-limitation (young) to co-limitation by both N and P (middle-aged), and then P-limitation in the oldest soils (Vitousek et al., 1997).

Previous studies of long-term chronosequences have focused on the aboveground ecosystem (e.g., Crews et al., 1995; Vitousek et al., 1997; Parfit et al., 2005) or on changes in soil chemistry with soil age (e.g., Walker and Syers, 1976; White et al., 1996; Vitousek et al., 1997). Given the focus on the above ground, little work has been done on root development with soil age across long-term chronosequences although one study found that roots were increasingly concentrated in surface (FH) horizons with soil age (Parfit et al., 2005). Two studies of soil microbial communities across long-term chronosequences evaluated microbial communities from the litter layer (Wardle et al., 2004b) or the litter layer and surface (0–5 cm) mineral soil (Williamson et al., 2005) of forest ecosystems. In both studies, microbial biomass, either in absolute terms (Wardle et al., 2004a,b) or relative to soil C (Williamson et al., 2005), generally decreased with soil age. Microbial community structure varied, including higher fungal to bacterial lipid ratios with increasing age in several of the chronosequences (Wardle et al., 2004b; Williamson et al., 2005).

This study is the first to investigate subsurface microbial communities across a long-term chronosequence. We use polar lipid fatty acid (PLFA) analysis to examine how soil microbial communities vary with depth and with inorganic and organic soil chemistry and plant productivity changes across a grassland chronosequence near Santa Cruz, California. Because previous studies have observed major changes in surface microbial communities either along disturbance and restoration gradients (McKinley et al., 2005; Allison et al., 2007a,b) or long-term chronosequence gradients (Wardle et al., 2004b; Williamson et al., 2005), we predicted that long-term changes in microbial communities would be most evident in surface soils and less evident in the subsurface.

2. Methods

2.1. Sample sites

The sample sites are located on tectonically-uplifted marine terraces in and near Wilder State Park, north of Santa Cruz, California. Located on minimally-eroding parts of the terraces, the sites were chosen with the criteria that mass loss by chemical depletion is much greater than losses resulting from physical erosion (White et al., 2008). The parent material for the soils is marine sediment primarily containing plagioclase feldspar, potassium feldspar, and quartz and derived from the Ben Lomond granite in the Santa Cruz Mountains (White et al., 2008).

This study builds on previous work at these same sites (Munster and Harden, 2002; Pinney et al., 2002; White et al., 2008, 2009; Maher et al., 2009) as well as previous work elsewhere on these terraces (e.g., Bradley, 1957; Aniku and Singer, 1990; Perg et al., 2001). Five terraces T1, T2, T3, T4, and T5 have been identified at Santa Cruz with ages of 65,000, 92,000, 137,000, 194,000, and 226,000 years old, respectively, as determined by cosmogenic nuclides (Perg et al., 2001). For this study, T4 was excluded due to recent anthropogenic disturbance, which resulted in the removal of up to 0.30 m of surface soil (White et al., 2008). The T1 site was in a park and had frequent addition of nutrients from pet waste and the microbial community was considered to be somewhat disturbed. Thus a full suite of microbial analyses was not performed on those samples.

Ca, K, Mg, and Na concentrations decreased with depth over the top meter of all soils (White et al., 2008). Base cations were also progressively depleted throughout the soil columns with increasing soil age as the primary minerals plagioclase (Ca and Na), potassium feldspar (K), and smectite (Mg) weather to form kaolinite and Fe oxides (White et al., 2008). As primary minerals were depleted, exchangeable base cations and pH also decreased (Pinney et al., 2002). In contrast, the total pool of Al and Fe in the soil profiles remained fairly constant with soil age although the depth distribution changed with progressive enrichment of Fe-oxide and kaolinite ($Al_2Si_2O_5(OH)_4$) in the upper 1–2 m of the older soils (White et al., 2008). Kaolinite also generally increased with depth over the upper 1 m (White et al., 2008).

The depth to the base of the A horizon in the soils varied from 0.25 to 0.87 m (Pinney et al., 2002) but was approximately 0.40–0.50 m at the sample sites. The depth to the base of the B horizon was at least 1.70 m (Pinney et al., 2002). The rooting depth varied from 0.45 to 0.75 m deep for T1, T2, and T3 and increased to 1.10–1.40 m depth for T5 (Munster and Harden, 2002). Fine and very fine roots dominated below 30 cm depth (Munster and Harden, 2002). Subsurface clay concentrations began to increase at 0.5–1 m depth forming a clay-rich, argillic horizon; clay concentrations reached a maximum between 1 and 1.5 m depth (Pinney et al., 2002; White et al., 2008). The argillic horizon results in a perched water table at T2, T3, and T5 during parts of the wet season (White et al., 2009). Soils on T1 through T4 have been classified as Typic Argixerolls and T5 has been classified as a Xeric Argialboll (Bowman and Estrada, 1980).

The climate is Mediterranean with a wet, cool winter and warm, dry summer. The mean annual temperature in Santa Cruz, California from 1948 to 2005 was 13.9 °C and the mean annual precipitation was 0.78 m (Western Regional Climate Center, 2007). Pollen data from a nearby marsh indicate grasslands were present on drier soils like the terraces from approximately 5000–12,000 years ago (Adam et al., 1981). Additionally, in many coastal prairie sites, endemic species, along with prairie soils, like the mollisols found at the Santa Cruz sample sites, indicate the coastal prairie ecosystem has been extant for hundreds of years (Heady et al., 1988). The sites were used as range land from the late 1700s through early 1970s (Wilder Ranch State Park, 2009) with aerial photographs confirming that the sampling locations were range land in the 1940s through early 1970s. The soils have not been grazed since the early 1970s when the area became a park. At present, the sites are part of the California coastal prairie ecosystem with annual European grasses currently dominating. Management practices include controlled fires (every 3–4 years) to prevent encroachment of chaparral species and encourage native coastal prairie species.

2.2. Sampling

Sites sampled for microbial community measurements were adjacent to sites characterized for bulk chemistry and mineralogy

(White et al., 2008) and pore water chemistry (White et al., 2006, 2009). Depth profile samples for polar lipid fatty acid (PLFA) analysis were collected as a single core from adjacent locations for T2, T3, and T5 during the dry season (June 2004) using a soil bulk density corer with removable internal brass rings. Between each sample, the rings were removed, cleaned with deionized water, and sterilized in the field using methanol. The samples were collected into sterile Whirl-Pak bags, transported on ice in a cooler, and stored at −20 °C until analysis. Samples were freeze-dried and subsampled for PLFA analysis and C and N analysis. Soil water contents were calculated from the difference of the pre- and post-freeze-dried samples. Additional information about the sites, including microbial biomass carbon, can be found elsewhere (Moore, 2008).

2.3. Aboveground plant biomass

The aboveground plant biomass data were collected on 10–15 different days at each terrace from October 2002 to November 2003 from a random area within approximately 20 m of geochemical and microbial sampling sites. Plant samples were collected by clipping one square meter of grass. After collection, samples were air dried and weighed to determine the biomass. Aboveground net primary productivity (ANPP) for each terrace was estimated by calculating the difference between the maximum and minimum grass biomass (Sala and Austin, 2000) collected between November 1, 2002 and October 31, 2003.

2.4. Carbon and nitrogen analysis

Unsieved samples of soil for C and N analysis were ground to a fine powder using a solvent-rinsed quartz agate mortar and pestle. Total C and N analyses were performed on duplicates of each sample using an elemental analyzer and are reported on a dry weight basis. Carbonate minerals were not detected in the terrace soils using x-ray diffraction. The total C concentrations measured on samples from this study are consistent with previously published organic C concentrations for these same terraces (Pinney et al., 2002). Thus we assumed that total C as measured in this study represents organic C.

2.5. Polar lipid fatty acid (PLFA) analysis

All glassware used for extraction and analysis was either solvent-rinsed (with methanol, chloroform/dichloromethane, and hexane) or ashed at 480 °C. Reagents were prepared using HPLC-grade solvents and 18.2 MΩ water. Before subsampling, samples were disaggregated using a solvent-rinsed ceramic mortar and pestle. Triplicates of each sample were extracted using a Dionex 200 Automated Solvent Extractor (ASE) in 33 ml cells following the method of Macnaughton et al. (1997) with slight modifications to the ASE cycle. Soil samples were mixed in a 5:1 ratio by weight with pre-extracted (using 2:1 methanol:dichloromethane) diatomaceous earth (VWR, Celite). Because the size of the microbial community decreased with depth, the mass of soil extracted was increased with depth below the surface. Five grams of soil were extracted from the 0.04 m interval, 8 g from the 0.25 m interval, 15 g from the 0.5 m and 0.75 m intervals, and 20 g from the 1 m interval. See Table 1 for the exact intervals.

The volume of the ASE cells not filled by sample was filled with ashed and pre-extracted Ottawa quartz sand. The ASE extractions were performed at 80 °C and 1200 psi with two 15-minute heating cycles and set to a 90% flush. The extraction was performed using a single-phase extractant (2:1:0.8 methanol/chloroform/0.05 M HK_2PO_4, pH 7.0). The ASE extractions were compared against extractions performed using a modified Bligh-Dyer extraction (Bossio and Scow, 1998; Macalady et al., 2000). Both methods yield similar lipid patterns and extraction efficiencies for a given mass of sample. However, the ASE extraction was more time efficient for extracting larger sample masses.

The $CHCl_3$ phase containing the total soil lipids was processed as described in Bossio and Scow (1998) and Macalady et al. (2000). Briefly, polar lipids were separated from the neutral and glycolipids by silica gel column chromatography. The polar lipids were transesterified using a mild alkaline methanolysis and then redissolved in 150 μl of hexane that contained a 19:0 fatty acid methyl ester (FAME) (Matreya, Pleasant Gap, PA) as an internal standard. The resulting FAMEs derived from polar soil lipids were stored under N_2 headspace at −20 °C until analysis. Samples were analyzed on a Hewlett–Packard 5972 GC–MS with a DB-5 column (J&W Scientific). For details of the GC–MS separation protocol, see the supplemental materials. Lipids were identified by comparing the

Table 1
Relative abundances of microbial groups from PLFA.

Soil	Top interval (m)	Bottom interval (m)	Midpoint depth (m)	Gram-positive bacteria (mole fraction)	Gram-negative bacteria (mole fraction)	Fungal (18:2ω9,12, mole fraction)	Fungal to bacterial lipid ratio	AMF (16:1ω5 mole fraction)	10Me18:0 (mole fraction)	10Me16:0 + 10Me18:0 (mole fraction)
T2	0.00	0.08	0.04	0.172 (0.002)	0.235 (0.003)	0.056 (0.008)	0.138 (0.021)	0.040 (0.003)	0.025 (0.003)	0.031 (0.003)
	0.23	0.30	0.27	0.211 (0.002)	0.242 (0.003)	0.005 (0.000)	0.011 (0.001)	0.030 (0.003)	0.016 (0.002)	0.028 (0.002)
	0.38	0.46	0.42	0.228 (*n*/a)[a]	0.243 (*n*/a)	0.003 (*n*/a)	0.006 (*n*/a)	0.021 (*n*/a)	0.023 (*n*/a)	0.037 (*n*/a)
	0.69	0.79	0.74	0.214 (0.002)	0.253 (0.006)	0.004 (0.001)	0.009 (0.002)	0.016 (0.002)	0.028 (0.004)	0.042 (0.004)
	0.91	0.99	0.95	0.230 (0.002)	0.314 (0.005)	0.003 (0.001)	0.006 (0.000)	0.015 (0.002)	0.029 (0.003)	0.048 (0.003)
T3	0.00	0.08	0.04	0.167 (0.001)	0.247 (0.003)	0.047 (0.005)	0.114 (0.013)	0.044 (0.003)	0.019 (0.001)	0.026 (0.001)
	0.23	0.30	0.27	0.174 (0.001)	0.193 (0.002)	0.027 (0.005)	0.073 (0.013)	0.030 (0.0002)	0.015 (0.001)	0.027 (0.001)
	0.48	0.56	0.52	0.231 (0.002)	0.190 (0.002)	0.006 (0.001)	0.013 (0.002)	0.027 (0.003)	0.024 (0.002)	0.051 (0.003)
	0.76	0.84	0.80	0.190 (0.002)	0.268 (0.006)	0.023 (0.008)	0.050 (0.018)	0.027 (0.002)	0.022 (0.002)	0.047 (0.002)
	0.99	1.07	1.03	0.221 (0.005)	0.248 (0.007)	0.022 (0.009)	0.048 (0.019)	0.025 (0.003)	0.017 (0.003)	0.051 (0.007)
T5	0.00	0.08	0.04	0.179 (0.001)	0.219 (0.001)	0.033 (0.003)	0.082 (0.007)	0.044 (0.001)	0.026 (0.002)	0.034 (0.002)
	0.20	0.28	0.24	0.177 (0.003)	0.304 (0.015)	0.011 (0.003)	0.023 (0.007)	0.025 (0.006)	0.017 (0.004)	0.031 (0.005)
	0.51	0.58	0.55	0.207 (0.001)	0.281 (0.004)	0.007 (0.002)	0.015 (0.004)	0.018 (0.0003)	0.028 (0.002)	0.055 (0.002)
	0.66	0.74	0.70	0.201 (0.002)	0.269 (0.009)	0.015 (0.004)	0.031 (0.008)	0.021 (0.004)	0.023 (0.001)	0.049 (0.003)
	0.99	1.07	1.03	0.248 (0.008)	0.234 (0.007)	0.013 (0.002)	0.027 (0.004)	0.018 (0.006)	0.027 (0.007)	0.060 (0.010)

$N = 3$ at each depth interval.
Numbers in parentheses are errors, which represent one standard deviation for single lipids (Fungal, 10Me18:0, AMF) and propagated error (square root of sum of squares of standard deviations) for multiple lipids.
[a] Only one replicate was run for this sample.

mass spectra to the NIST98 database and by comparison to the CP Mix FAME lipid standard (Matreya, Pleasant Gap, PA). Lipid concentrations were calculated using the internal 19:0 FAME standard and reported per mass of dry soil. Lipid nomenclature follows the A:BwC form, where A is the number of carbons in the lipid, B indicates the degree of saturation, and C indicates the position of double bonds with respect to the methyl end of the lipid; a number preceding A, e.g., 10Me, indicates a methyl group on the tenth carbon from carboxyl end of the lipid (Frostegard et al., 1993). The prefix "cy" indicates a cyclopropyl group.

Certain lipids (biomarkers) can serve as indicators of specific microbial groups. Branched saturated (iso- and anteiso-) lipids including i15:0, a15, i16:0, i17:0 and a17:0 have been found to be indicators of Gram-positive bacteria (Zelles, 1997). Lipids used as indicators of Gram-negative bacteria in this study were 16:1ω9, 18:1ω9, cy17:0, and cy19:0 (Zelles, 1997). Me16:0 and Me18:0 lipids indicate the presence of actinobacteria (Zelles, 1997), especially in soil environments not expected to host a large proportion of sulfate-reducing bacteria (Macalady et al., 2000). The 18:2ω9,12 lipid indicate fungi (Zelles, 1997). For more details on fungi and the 18:2ω9,12 lipid, see the supplemental materials. The 16:1ω5 lipid, tentatively identified as the single double-bonded 16 chain C eluted after 16:1ω9, indicates arbuscular mycorrhizal fungi (Olsson, 1999). The bacterial lipids included in the fungal (18:2ω9,12) to bacterial ratio calculation were those listed above as indicators of Gram-positive bacteria and Gram-negative bacteria along with the Me18:0 lipid.

2.6. Statistical analyses

Differences in soil and microbial parameters were assessed using *t*-tests in StatPlus:mac2008 (AnalystSoft, 2008). Lipid data were analyzed by ordination analysis performed with the statistical software package R (R Development Core Team, 2007) with the vegan library (Oksanen et al., 2007). Lipids included in ordination analyses and the total PLFA biomass presented were those common to all sample intervals (number of lipids $n = 20$ from 0 to 1.07 m depth). For ordination, each lipid concentration was converted to a fractional abundance of the total PLFA biomass (nmol g^{-1}). Correlation matrices were used for the PCA and RDA analyses. The environmental fit function in R was used to test the correlation between environmental variables and the principal component axes. Environmental variable concentrations are from the depth interval most similar to the microbial sample depth except for two intervals. For the T2 surface sample, CaO and P were extrapolated from the next interval down, K_2O was set as the same as the next interval down, and Fe_2O_3 and kaolinite were calculated as the average of the two intervals below the surface. Values for all environmental variables for the T5 0.75 m sample were set to the average of the samples just above and below this interval. The amount of variance accounted for along each RDA axis, and the statistical significance of that variance (calculated using ANOVA) were tested both manually and with the automated step function, which used Akaike's information criterion (Oksanen et al., 2007), to maximize the variance explained with the fewest number of environmental variables.

3. Results

3.1. Variations with depth

Soil chemistry parameters that decreased with depth over the top meter were P and P availability as estimated by the P to Fe ratio (Fig. 1) and C, N, and the C to N ratio (Fig. 2). The following microbial parameters decreased with depth: PLFA biomass (Fig. 3), the PLFA to C ratio (Fig. 3), and number of different lipids detected (Table S4). C and N at the surface are higher than concentrations at ~1 m by a factor of 2–12 whereas the ratio between the surface concentration and 1 m depth for PLFA biomass is larger, 30–>100. Following the trend of rapid decrease in PLFA concentrations below the surface, the lipid concentration for a 25 g sample extracted from the ~1.25 m interval for T2 was approximately 0.01 nmol per g, indicating an extremely small microbial community deeper in the soils. PLFA biomass and C ($R^2 = 0.92$, Fig. S1), PLFA biomass and N ($R^2 = 0.88$, Fig. S2), and subsurface PLFA biomass and P to Fe ratios ($R^2 = 0.87$, Fig. S3) were all highly correlated. PLFA biomass and water content were not strongly correlated ($R^2 = 0.30$, not shown).

Soil water content increases with depth in all soils (Fig. 2). The fraction of lipids associated with Gram-positive and actinobacteria groups generally increases with depth (Table 1). The proportion of lipids associated with Gram-negative bacteria does not show a consistent trend with depth (Table 1). While the ratio of fungal to bacterial lipids is highest at the surface in each soil, it reaches a minimum at 0.5 m and then increases for T3 and T5 (Table 1, Fig. 3). The fraction of AMF lipid was highest at the surface and lower, though with no trend, in the subsurface (Table 1). PCA and

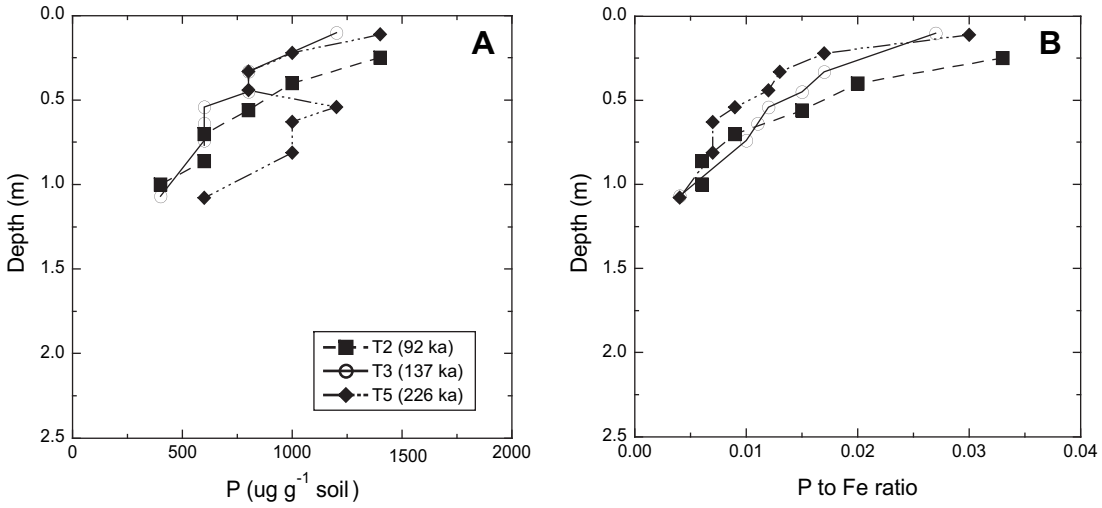

Fig. 1. Concentration versus soil depth for (A) P and (B) P to Fe ratio, a proxy of P availability. Fe concentration data were published in White et al. (2008). The data for these parameters are listed in Table S1.

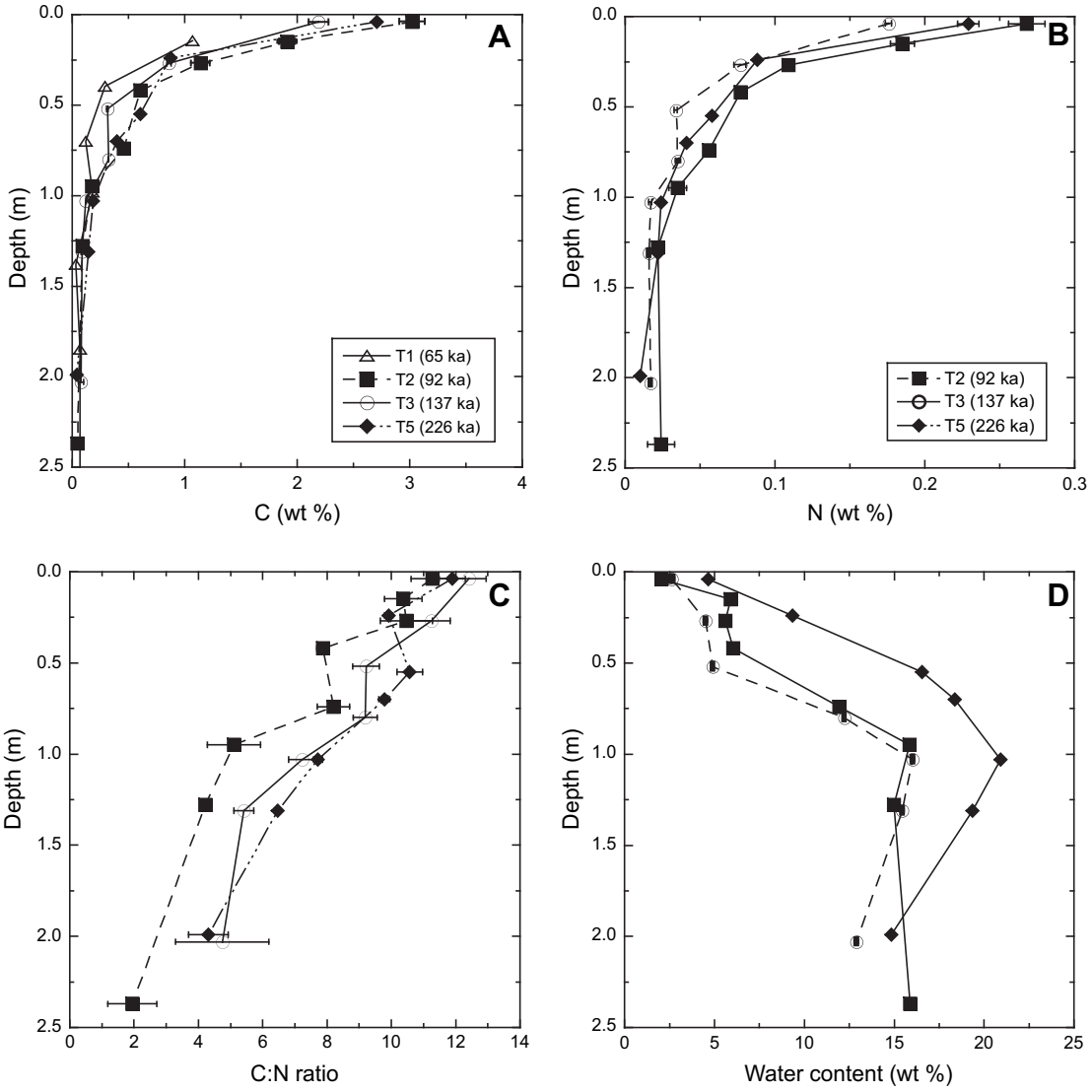

Fig. 2. Concentration versus depth in bulk soil for (A) carbon, (B) nitrogen, (C) carbon to nitrogen ratio, and (D) water content. Error bars for (A) and (B) represent one standard deviation; error bars for (C) represent the propagation of uncertainty from the standard deviations of (A) and (B). The data for these parameters are listed in Table S2.

RDA of microbial community data indicate community structure changes markedly with depth.

3.2. Variations with age

Variations with age fit into three main patterns. First, most parameters that decrease in concentration with depth also decrease in concentration with soil age. Parameters that fit this first pattern are P concentrations (higher in the upper 0.5 m for T2 than for T3 or T5, Fig. 1), P availability as estimated by the P to Fe ratio (Fig. 1), C and N from T2 to T3 and T5 (Fig. 2), and subsurface PLFA concentrations and the PLFA to C ratio (Fig. 3). Subsurface PLFA biomass and PLFA to C ratios for samples $\geq$0.5 m depth are higher at T2 than at T3 and T5 (t-tests, $P \leq 0.05$, data not shown) due to a more rapid decline with depth in the older soils (Fig. 3). The fraction of the total PLFA biomass contributed by the fungal biomarker lipid decreases with soil age in the surface samples. Second, ANPP (Table S1) as well as C and N concentrations (Fig. 2) increase from T1 to a maximum at T2 and then decrease for T3 and T5. Third, some of the parameters that increase in concentration with depth also increase with soil age. Soil water contents for samples $\geq$0.5 m depth increase with soil age, particularly in T5, which has water contents 1.3–2.8 times higher than T2 and T3 (Fig. 2). The C to N and fungi to bacteria ratios in samples from >0.5 m also increase with soil age. One parameter that did not increase with depth but did increase with soil age is surface PLFA biomass, which increases somewhat from T2 and T3 to T5 (t-tests, $P \leq 0.05$, data not shown). In the subsurface, the fungal fraction and fungal to bacterial lipid ratio decline sharply for T2 and remain low to 1 m depth. In contrast, for T3 and T5, the fungal fraction and fungal to bacterial lipid ratio initially decrease from the surface down to 0.5 m but then increase in the 0.75 and 1 m intervals (Table 1, Fig. 3). Slight increases in subsurface Gram-positive and actinomycete bacterial lipids with soil age are not significant (Table 1, t-tests >0.05, data not shown). Gram-negative and AMF lipids show no pattern with age.

3.3. Microbial community structure

PCA ordination reveals differences in microbial community structure among samples, with 46% of the variability explained by PC1 and 19% of the variability explained by PC2 (Fig. 4). A PCA ordination performed with lipids common to all samples collected

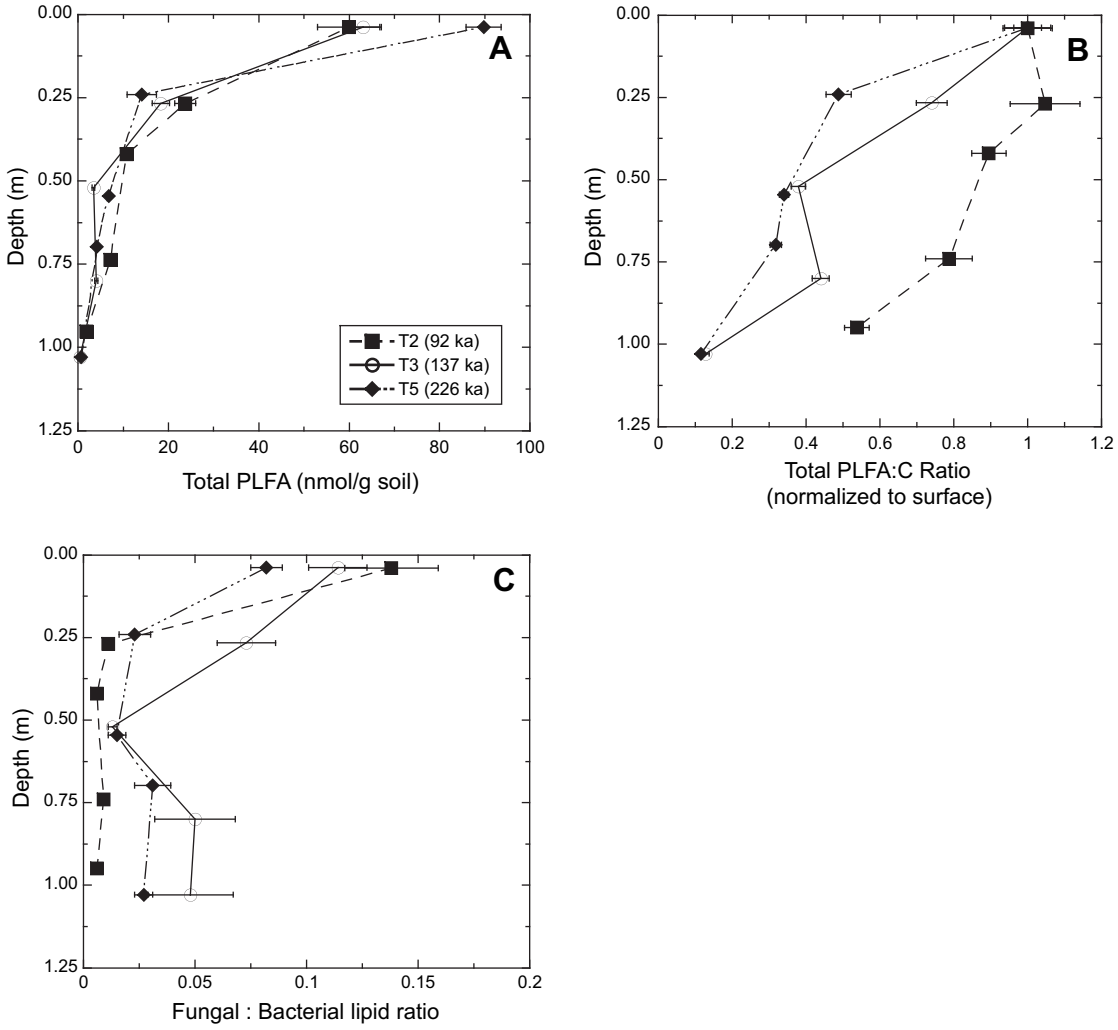

Fig. 3. Concentration versus depth for (A) total PLFA, (B) PLFA to C ratio with values normalized to the surface value for each soil, and (C) fungal to bacterial lipid ratio. Error bars represent the propagation of uncertainty from the errors for each component in the sum or ratio. Data for (A) are listed in Table S4.

from ≤0.75 m ($n = 39$) displays nearly identical pattern to the smaller subset (results not shown). Since some of the soil lipids could potentially originate from fine plant roots that we were unable to remove during soil processing, we also performed PCA on a subset of lipids (≤0.75 m, $n = 32$) excluding PLFAs typically found in plants (16:1ω9, 16:1ω5, 16:0, 18:2ω9,12, 18:1ω9c, 18:1ω9t, 18:0). PCA results (not shown) were nearly identical whether typical eukaryote PLFAs were included or not.

Results of the environmental fit indicated that correlations with Fe_2O_3 concentrations and age were not statistically significant but all other variables are significantly correlated ($p < 0.05$, Table 2). Environmental fit results indicate that changes in C, N, and P_2O_5 concentrations, the C to N ratio, and depth are closely aligned (≥0.85 or ≤−0.85) with PC1 (Table 2). Environmental factors closely aligned with PC2 (≥0.85 or ≤−0.85) are CaO, K_2O, and kaolinite (Table 2).

RDA results strongly suggest that C concentrations, CaO concentrations, and soil water content account for nearly all the variance accounted for in the first two axes of the PCA (Fig. 5). RDA1, RDA2, and RDA3 accounted for 44%, 16%, and 5% of the variance, respectively. The sum of the variance accounted for along RDA1, 2, and 3 equals the sum of the variance accounted for along PC1 and PC2. The statistical significance of the variance correlated with the environmental variables is $p < 0.001$ for C and CaO and $p < 0.05$ for water content. If water content is excluded from the RDA, then 41% and 15% of the variance are accounted for along RDA1 and RDA2. Thus water content accounts for much less of the variance than either C or CaO. When N concentrations are substituted for C concentrations, the variance accounted for and the significance are almost identical. When K_2O or kaolinite concentrations are substituted for CaO concentrations, the variance accounted for along RDA2 is smaller with reduced statistical significance.

4. Discussion

4.1. Depth trends

Maximum P concentrations and availability at the surface (Fig. 1) suggest that much of the non-occluded P is concentrated in plant litter and SOM in all the soils. C and N concentrations decrease with depth because plant communities are the primary source of C and N to soils (Wardle et al., 2004a). Overall decreases in PLFA biomass with depth in the Santa Cruz soils correspond with decreasing C, N, and P availability (Figs. 1,2,4). The high correlation between PLFA biomass and C for all samples (Fig. S1), PLFA biomass and N for all samples, and PLFA biomass and P availability for subsurface samples (Fig. S3) likely occurs because much of the

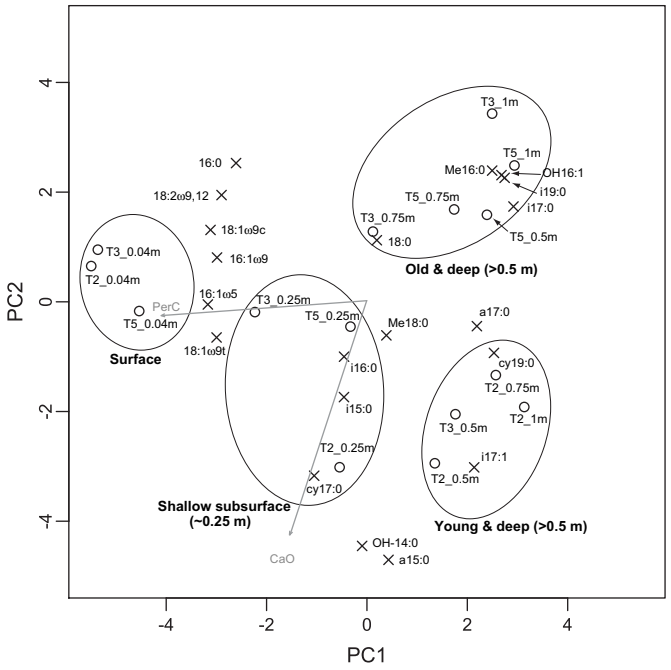

Fig. 4. Results for principal components analysis (PCA) of the PLFA data. The number following each soil designation is the approximate midpoint depth of the soil sample. Loading scores are plotted for both the sites (circles) and the individual lipids (X's). Note that the lipid scores are multiplied by 10 for display purposes. The PCA accounted for 46% of the variance in community composition along PC1 and 19% along PC2. The environmental variables that correspond best with PC1 (C concentrations (PerC)) and PC2 (CaO concentrations) are plotted as arrows. Loading scores for other environmental variables can be found in Table 2.

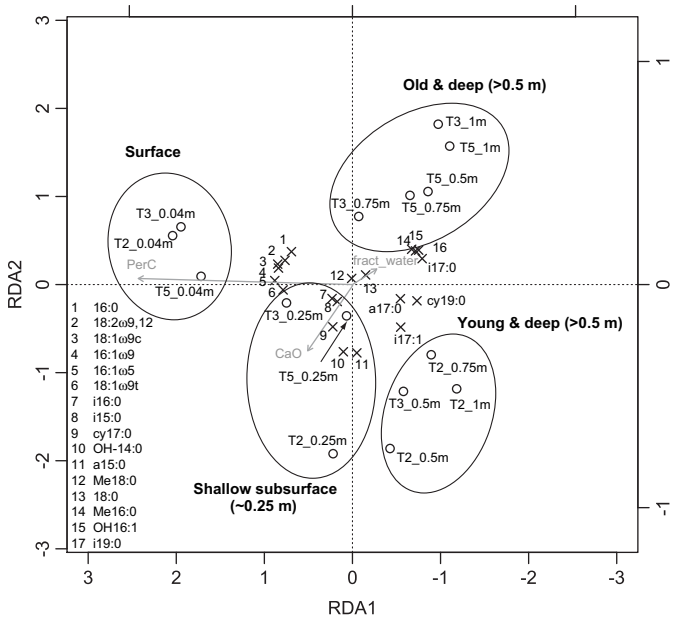

Fig. 5. Results for redundancy analysis (RDA) of PLFA data. Symbols are the same as Fig. 4 although lipid loading scores are not multiplied by 10 in this figure. Note that the RDA1 axis is reversed for ease of comparison with the PCA results. The RDA accounted for 44% of the variance in community compositions along RDA1 and 16% along RDA2. The statistical significances were $p < 0.001$ for C (PerC) and CaO and $p < 0.05$ for water content (fract_water). The arrows are scaled to the proportion of variance explained along each RDA axis (with fract_water corresponding with RDA3).

microbial community is heterotrophic, and thus expected to decrease in size as C, N, and P availability decrease. In addition to smaller biomass with depth, the PLFA to C ratio declines with depth (Fig. 3) indicating that subsurface microbial communities are less efficient at utilizing or have less access to SOM present deeper in soil profiles. Significant decreases in C, N, and PLFA biomass with depth at Santa Cruz are similar to other studies of grassland soil microbial communities as a function of depth (Blume et al., 2002; Fierer et al., 2003; Allison et al., 2007b) as are the decreases in P availability with depth (Allison et al., 2007b).

While water content increases with depth in all the soils, due in part to increased clay contents with depth, the lack of a significant correlation between PLFA biomass and water content suggest that water content is not a driver of microbial biomass during the dry season when the largest differences in water contents are observed.

Table 2
Environmental fit loading scores.

Environmental variable	PC1[a]	PC2	R^2	P (significance)
C	−1.00	−0.06	0.86	<0.001
PLFA biomass	−1.00	−0.05	0.81	<0.001
N	−0.99	−0.16	0.83	<0.001
P_2O_5	−0.96	−0.27	0.54	<0.05
C:N	−0.94	0.33	0.68	<0.001
P_2O_5:Fe_2O_3	−0.78	**−0.63**	0.68	<0.01
CaO	−0.39	**−0.92**	0.80	<0.001
K_2O	−0.33	**−0.94**	0.70	<0.01
Kaolinite	0.50	0.87	0.79	<0.001
Water content	0.71	0.71	0.86	<0.001
Depth	0.87	0.50	0.86	<0.001

[a] Environmental variable fits sorted by the correlation with PC1. Variables with correlation ≥0.50 for PC2 are underlined and variables with loading scores ≤−0.50 for PC2 are displayed in bold type. Correlations with soil age and Fe_2O_3 were not reported because the relationships were not statistically significant.

Previous work yields conflicting results regarding the correlation between seasonality, including water content, and microbial biomass with some studies observing no correlation (Bossio and Scow, 1998; Blume et al., 2002; Waldrop and Firestone, 2006) but other studies finding a correlation between water content and biomass (Van Gestel et al., 1992; Bardgett et al., 1999). In the wet season at Santa Cruz, water contents are more evenly distributed throughout the soil profile resulting in higher water contents at the surface and similar water contents in the subsurface in comparison to the dry season (White et al., 2009). Thus if water content and biomass are correlated, then microbial biomass will increase most at the surface, which has the lowest water content in the dry season, further accentuating the observed pattern of the maximum microbial biomass at the surface.

Decreasing microbial community biomass with depth is accompanied by a shift in microbial groups. Gram-positive bacteria and actinobacteria increase as a fraction of the microbial community (Table 1), which has also been seen in other studies (Fritze et al., 2000; Blume et al., 2002; Fierer et al., 2003; Potthoff et al., 2006; Allison et al., 2007b). Previous investigators suggested that this increase is a response to declines in soil nutrient availability (Fritze et al., 2000; Fierer et al., 2003). The overall decline in the fungal portion of the microbial community with soil depth seen at Santa Cruz (Fig. 4) also matches well with results from previous studies (Fritze et al., 2000; Ekelund et al., 2001; Fierer et al., 2003). However, at T3 and T5, the fungal portion of the population increases from a minimum at 0.5 m to a secondary maximum at 1 m (Fig. 4). A similar trend was reported, but not discussed, by Fierer et al. (2003). Reasons for this pattern remain to be elucidated in future work.

Much of the variance in microbial community structure corresponds with sample depth with the surface samples were found to the left side of PC1 and progressively deeper samples to the right. Because differences in microbial community structure with depth can be explicitly connected to concentration gradients in

environmental variables, depth is not discussed further as an environmental variable. The alignment of C, N, P, and C to N gradients with PC1 (Table 2) suggests that the change in community structure with depth is most strongly related to SOM availability. The close alignment of the C gradient, assumed to be equivalent to SOM, with RDA1 (Fig. 5) further confirms this relationship. RDA demonstrates that changes in water contents only account for a small percentage of the variance in microbial community structure suggesting that the more even water distribution in the wet season would only have a slight effect on community structure.

The variance of microbial community structure accounted for along PC1 (46%) was similar to other grasslands: 42% for southern California grassland (Fierer et al., 2003), and 56% for Illinois prairie (Allison et al., 2007b). Also consistent with this study, shifts in community structure frequently correlate with SOM gradients, although other environmental variables can also play a role, e.g., in Illinois prairie soils where Ca and P corresponded with PC1 (Allison et al., 2007b). Some of the same lipids, including 18:1w9c and cy19:0, drive the differentiation of microbial community structure with depth in this and previous studies (Fritze et al., 2000; Fierer et al., 2003; Allison et al., 2007b).

4.2. Age trends

Increases in subsurface water contents are consistent with higher concentrations of the finer-grained secondary minerals in older soils. The overall decrease in P availability with soil age (Fig. 1) matches well with previous studies where an increasing percentage of P becomes occluded with soil age, i.e., not readily available to plants or the soil microbial community, due to sorption and coprecipitation with Fe-oxides (Walker and Syers, 1976; Crews et al., 1995). Not only does Fe increase with age in the bulk soil, but also the concentration of Fe-rich nodules, which have higher P concentrations than bulk soil, increases with age in the top meter of soil (Schulz et al., in press).

The initial increase in ANPP from T1 to T2 and then the general decrease with age to T3 and T5 is similar to trends observed in forested chronosequences (Vitousek et al., 1997; Wardle et al., 2004b). Integrated C and N concentrations over the top 0.5 m display the same pattern as ANPP and match well with previous forest (Crews et al., 1995) and grassland (Harden, 1987) chronosequence studies. Declining ANPP for the older soils results in lower organic matter input to the soils and explains lower C and N concentrations. While C and N concentrations are highest for T2 and lower for T3 and T5, surface soil C to N ratios do not show a trend with age, as was the case for ≥20,000 year-old soils of two forested chronosequences (Crews et al., 1995; Williamson et al., 2005). Higher C to N ratios in T3 and T5 samples from ≥0.5 m (Fig. 2) likely indicate a reduction in SOM quality with similar trends observed in older soils from forest chronosequences (Crews et al., 1995; Williamson et al., 2005). The observed trends in ANPP, C, and N with soil age match reasonably well with results for long-term forested chronosequences and indicate similarity in ecosystem processes between grasslands and forests.

Observed shifts in PLFA biomass, the PLFA to C ratio, and microbial community structure as a function of soil age occur predominantly in the subsurface (Table 1, Figs. 2,4). It is possible the history of grazing, although grazing last occurred approximately 35 years previous to sampling, is partially responsible for the similarity of microbial community structure in the surface soils. PLFA biomass and PLFA to C ratios decrease more rapidly compared to the surface maximum in the T3 and T5 soils compared to T2 (Fig. 4). As with the depth trends, reduced PLFA to C ratios as a function of soil age indicate less accessible SOM. When PLFA to C ratios was normalized by the surface sample value, subsurface PLFA to C ratios decrease from T2 to T3 to T5, except for 1 m depth where T3 and T5 are the same within uncertainty (t-tests, $P \leq 0.05$, data not shown). Our data suggest that subsurface microbial communities in older soils are less efficient at utilizing the organic matter present in the system.

Mineral weathering and pedogenic processes are likely the primary causes of lower subsurface PLFA biomass and shifts in microbial community structure with soil age (discussed below). Pedogenesis has indirect effects through declining ANPP with soil age as seen in this and numerous other studies (e.g., Crews et al., 1995; Vitousek et al., 1997; Williamson et al., 2005) and to a small degree through increased subsurface water contents, which are consistent with higher concentrations of finer-grained secondary minerals in the older soils. Direct effects arise from reductions in base cation concentrations and pH. Other grassland studies have also found that decreasing base cation concentrations and pH result in lower microbial biomass (Grayston et al., 2004; Allison et al., 2007b). Smaller subsurface PLFA biomass also correlates well with available P. Thus availability of P in soil plays an important role, not only for ANPP, but also for microbial processes where P-limitation has been shown to reduce microbial activity (Cleveland et al., 2002, 2004).

Although Gram-positive bacteria and actinobacteria increased with depth, they did not increase with soil age. The absence of a trend with soil age may indicate that either the increase with depth is not due to nutrient limitations as has been proposed by Fierer et al. (2003) and others or that the drivers of nutrient limitations with depth are different than the drivers of limitations with soil age and increasing nutrient limitation. The fungal fraction and fungi to bacteria lipid ratio were the only microbial group lipids to exhibit clear changes with soil age with a decrease in surface samples with soil age and an increase in the subsurface (≥0.5 m) with soil age (Table 1, Fig. 3). Lower fungi to bacteria ratios in older soil(s) was observed in two of seven forest and shrubland chronosequences (Wardle et al., 2004b; Williamson et al., 2005). The fungi to bacteria ratio may not be directly comparable between grasslands and forests because microbial communities in grasslands soils tend to be more dominated by bacteria, whereas fungi tend to dominate in forest soils (Bardgett et al., 1999; Fierer et al., 2003; Grayston et al., 2004; McKinley et al., 2005; Allison et al., 2007b). However, the fungal fraction varies along nutrient gradients in grassland soils with the fungal fraction of the surface microbial communities increasing from managed (fertilized) to unmanaged (nutrient-poor) grassland soils as nutrients become more limited (Bardgett et al., 1999; Grayston et al., 2004). Nutrient limitations are strongly suggested for all surface soils at Santa Cruz where the fungal to bacterial lipid ratios are ≥2 times larger than reported ratios from other grassland studies (Bardgett et al., 1999; Grayston et al., 2004). Additionally, increased fungal fractions and fungal to bacterial lipid ratios in T3 and T5 subsurface samples (≥0.5 m) indicate nutrient limitations in the older soils. The increases in the fungal portion of the subsurface community with soil age occur despite seasonal saturation, an environmental condition that resulted in decreased fungi in similar soils (Drenovsky et al., 2004).

PCA and RDA analysis of microbial community structure show that surface communities are similar across the chronosequence and that shifts in the microbial community structure at Santa Cruz occur predominantly in the subsurface, particularly ≥0.5 m (Figs. 4, 5). In Fig. 5, moving from bottom to top along PC2 corresponds with younger to older subsurface microbial communities. RDA results indicate that pedogenic processes, as exemplified by CaO concentrations, account for nearly all of the variance along RDA2 (Fig. 5). The decrease in CaO with soil age due to increased

depletion of plagioclase serves as a proxy for pedogenic processes since the environmental fit parameter for kaolinite is approximately 180° from the CaO fit. The mechanism responsible for the effect of Ca and pedogenesis on Santa Cruz soil microbial communities is unclear but is likely related to lower soil pH as Ca and other base cations are depleted from the soil by weathering. Relationships between lower Ca concentrations, lower soil pH, smaller microbial biomass, and changes in microbial community structure have been observed in grassland subsurface soils across a restoration chronosequence (Allison et al., 2007b). Additionally, recent studies have highlighted pH as an important controlling variable for microbial community structure in soils across different ecosystems and soil types (Fierer and Jackson, 2006; Lauber et al., 2008). Given that the changes in Ca and pH at Santa Cruz are a function of soil age, about one-third of the variance in soil microbial community structure across the chronosequence accounted along the first two axes of the PCA and RDA can be attributed to increased duration of pedogenic processes, including primary mineral dissolution and secondary mineral formation, in the older soils. Unlike previous studies of microbial community structure that focused on younger chronosequences or nutrient gradients, we are able to relate the remaining variance in PLFA patterns ($\sim$19%, PC2 and RDA2) to soil chemistry gradients that vary primarily with soil age. Increased soil age and pedogenic duration are the most likely reason for the success of this study in correlating PC2 and RDA2 to environmental gradients.

4.3. Feedbacks between plant and soil microbial communities and soil chemistry with soil age

Our data indicate that there are feedbacks between soil microbial communities, plant communities, and duration of pedogenic processes at the Santa Cruz chronosequence. The ecosystem, including both the microbial and plant communities, shifts with soil age. In the younger Santa Cruz soils where base cations and P are more available, ANPP and microbial community biomass are higher. Soil microbial communities in more nutrient-rich soils like in the subsurface of T2 have a higher proportion of bacteria with "leaky" nutrient cycling because microbial growth happens in rapid bursts during which excess nutrients are lost from the soil (Bardgett et al., 2005). The increased fungal component in more nutrient-poor soils, as is observed in the subsurface of T3 and T5, may help retain nutrients within the soil more effectively with less nutrient loss due to leaching because nutrient cycling is slower (Wardle et al., 2004a; Bardgett et al., 2005). A microbial community with relatively more fungi also may be more effective at accessing the less accessible nutrient pools in the older soils, particularly P (Blum et al., 2002; Bardgett et al., 2005; van der Heijden et al., 2008).

Lower P availability was also proposed to slow nutrient cycling and N fixation in a previously studied long-term granitic grassland chronosequence (Baisden et al., 2002) and also can limit microbial activity (Cleveland et al., 2004). As nutrient cycling slows, fewer nutrients are readily available for plants and plant productivity decreases (Vitousek et al., 1997), which then results in lower organic matter input to the soil and biomass. With fewer nutrients available for plant growth, plant litter quality is reduced, further contributing to the slowing of nutrient cycling (Crews et al., 1995; Carney and Matson, 2005).

Additionally, as the overall SOM pool becomes more recalcitrant, organic ligands including humic and fulvic acids can become an important source of nutrients to the soil microbial community. Formation of Al- and Fe-rich horizons in subsurface soils, like those seen at Santa Cruz, is partially due to microbial consumption of organic ligands complexed with Al and Fe (Lundström et al., 2000). The consumption of ligands releases the Al and Fe to solution resulting in the precipitation of secondary minerals composed of these insoluble elements. Precipitation of the secondary minerals affects soil hydrology (White et al., 2009) and the capacity of the soil to retain C (Torn et al., 1997). Downward transport of Al- and Fe–organic complexes has been demonstrated to occur in grasslands similar to Santa Cruz (Masiello et al., 2004) as well as in podzolic forest soils (Lundström et al., 2000).

At Santa Cruz, longer duration of pedogenesis with soil age results in the separation of biological and geochemical cycling as can be seen most clearly at T5 where biological cycling of elements is concentrated in the upper meter and geochemical cycling and active weathering of primary minerals is found deeper than 1 m (White et al., 2006). While the separation of biological and geochemical cycling commonly occurs in tropical and subtropical soils, evidence from Santa Cruz indicates this process also can be important in old, slowly eroding landscapes in temperate climates. In systems like the older Santa Cruz soils where the biological and geochemical cycling are largely separated, most nutrients are located in the biological or SOM pools. The biological and SOM pools are concentrated at and near the soil surface and are tightly cycled. Perturbation, or disturbance, can break the nutrient cycle and results in the loss of scarce nutrients from the system. As a result, these types of ecosystems are likely to be particularly sensitive to perturbations and restoration of their original state after perturbation may prove to be a difficult or impossible task (Walker et al., 2001).

4.4. Conclusions

This study, the first to examine variations in subsurface soil microbial communities across a long-term grassland chronosequence, finds that the variations occur primarily at depths $\geq$0.5 m for both the fungal component of the microbial community and the structure of the entire microbial community. Additionally, our study is the first to correlate microbial community shifts not only with environmental variables that change as a function of soil depth but also across another environmental gradient – varying soil chemistry as a function of soil age. Our results highlight the likelihood that slowly eroding, older landscapes are sensitive to perturbation in temperate regions as well as in tropical and subtropical regions.

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Appendix. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.soilbio.2009.09.015.

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