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Linking microbial community composition and soil processes in a California annual grassland and mixed-conifer forest

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Abstract. To investigate the potential role of microbial community composition in soil carbon and nitrogen cycling, we transplanted soil cores between a grassland and a conifer ecosystem in the Sierra Nevada California and measured soil process rates (N-mineralization, nitrous oxide and carbondioxide flux, nitrification potential), soil water and temperature, and microbial community parameters (PLFA and substrate utilization profiles) over a 2 year period. Our goal was to assess whether microbial community composition could be related to soil process rates independent of soil temperature and water content. We performed multiple regression analyses using microbial community parameters and soil water and temperature as X-variables and soil process rates and inorganic N concentrations as Y-variables. We found that field soil temperature had the strongest relationship with CO_2 production and soil NH_4^+ concentration, while microbial community characteristics correlated with N_2O production, nitrification potential, gross N-mineralization, and soil NO_3^- concentration, independent of environmentalcontrollers. We observed a relationship between specific components of the microbial community (as determined by PLFA) and soil processes,particularly processes tightly linked to microbial phylogeny (e.g. nitrification). The most apparent change in microbial community composition in response to the 2 year transplant was a change in relative abundance of fungi (there was only one significant change in PLFA biomarkers for bacteria during 2 years). The relationship between microbial community composition and soil processes suggests that prediction of ecosystem response to environmental change may be improved by recognizing and accounting for changes in microbial community composition and physiological ecology.

Introduction

Soil microbial communities mediate many biogeochemical processes that are central to ecosystem functioning, including carbon (C) mineralization to CO_2, nitrogen (N) cycling, and trace gas production and consumption. Despite the centrality of microbes, scientists often study ecosystem functioning without explicit reference to the microbial communities carrying out soil processes. The potential for rapid microbial growth, and the high degree of diversity and genetic exchange in microbial systems, has led to a common assumption that microbial activity does not constrain the processes involved in ecosystem

nutrient transfer and transformation (Meyer 1994; Andren et al. 1999). Models of ecosystem functioning have thus been based on assumptions that may only work well under pseudo-equilibrium conditions. For example, the assumption of first-order kinetics common in carbon cycle modeling may work well at equilibrium, but its utility in predicting soil process rates may be compromised in systems undergoing environmental transition or disturbance (Balser et al. 2002b). In order to predict the transient responses of ecosystems to changing environmental conditions, we may need a detailed understanding of the relationship between microbial community response and changes in soil processes such as decomposition and nitrogen mineralization (Schimel 2001).

Studies specifically designed to assess the relationship between community composition and process rates have led to a growing realization that microbial community composition can play a critical role in ecosystem functioning independent of environmental parameters (Schimel 1995; Schimel and Gulledge 1998; Cavigelli and Robertson 2000). In recent years there has been new emphasis on seeking ways to quantitatively link microbial community composition and soil processes as interest grows in understanding how microbial diversity might be related to ecosystem functioning (Schimel and Gulledge 1998; Cavigelli and Robertson 2000; Balser et al. 2002b; Waldrop and Firestone 2004). However, there have been few studies that quantitatively link microbial community characteristics and soil process rates (Waldrop and Firestone 2004). This is due in part to the challenges inherent in microbial community study; communities are exceedingly complex and their structure and function are difficult to quantify let alone connect (Balser et al. 2002b). Microbial ecological studies relying on gene-based techniques can provide highly detailed community-taxonomic data sets. However, ecosystem ecology studies do not generally have as their goal complete characterization of the community, but rather parameterization of the microbial community in a way that can be related to soil processes. Microbial biomass has been by far the most commonly used parameter, yet in fact provides very little information about the microbial community (Balser et al. 2002b). While signature lipid biomarker analysis and substrate utilization have been increasingly used to represent aspects of microbial community structure and function in many recent ecosystem studies, few studies have assessed the relationship of these parameters to ecosystem functioning or soil processes. Thus the relevance of substrate utilization and other microbiological characteristics (e.g., microbial biomass, diversity) to soil process rates is the subject of some controversy (Konopka et al. 1998; Balser et al. 2002a, b). One of the goals of this study was to assess the value of different microbial community characteristics (biomass, substrate utilization, diversity indices, and lipid profiling) as predictors of soil processes (N mineralization, nitrification, CO_2 and N_2O flux).

We transplanted soil between a grassland and conifer forest ecosystem, incubating intact cores *in situ* for 2 years, and quantified soil process rates and microbial community parameters in cores remaining in place and in those moved to a new climate/ecosystem. We used these data to investigate the

importance of microbial community characteristics in the response of soil processes to transplant. The experimental design allowed us to assess the importance of microbial community composition independent of soil temperature and water content in controlling soil processes through multiple regression analysis. In addition, there are few studies of microbial community structure that last beyond a growing season. Most studies of microbial ecology are carried out over a short time period (weeks to months). This provides limited insight into the longer-term dynamics of microbial communities. In this study we sampled over the course of 2 years. Our data allow us to assess the longer-term response of the soil microbial community to a disturbance (e.g., soil transplant).

Materials and methods

Field sites

The study was conducted at two locations along an elevation gradient on the western slope of the Sierra Nevada (at roughly 400 and 1200 m a.s.l.). The soils at both sites are similar in parent material, age, topography (relief, slope, sun angle), but experience a different annual climate (Jenny et al. 1949). Temperature and precipitation data, and soil properties are summarized in Table 1. The two sites were chosen to allow us to quantify the response of two different soil microbial communities to a change in climate (as represented by change in elevation).

The lower-elevation ecosystem is classified as a blue oak annual grassland savanna, composed primarily of a *Quercus douglasii* overstory (~60% cover) and an annual grassland understory. The most abundant species in the open grassland areas are *Bromus mollis*, *Hordeum hystrix*, and *Avena barbata* (Balser, *unpublished data*). The climate is Mediterranean, with cooler temperatures and precipitation concentrated from November to February. In open grass areas at the lower elevation soil maximum daily temperatures often exceed 45 °C during the summer, and are usually below 10 °C during the winter (Huenneke and Mooney 1989). The soil is from the Fallbrook soil series, of the subgroup Mollic Haploxeralfs, and is formed from weathered granodiorite parent material (Trumbore et al. 1996). The surface organic horizon overlying the mineral soil persists throughout the year, ranging in depth from 1 to 2 cm. Most plant roots and microbial biomass occur in the upper 10 cm of the mineral soil, under the grass canopy type (Huenneke and Mooney 1989; Jackson et al. 1988).

The higher-elevation site is located within the Sierra Nevada National Forest near Shaver Lake, CA. The forest is a mature mixed-conifer stand comprised of incense cedar (*Calocedrus decurrens*), ponderosa pine (*Pinus ponderosa*), manzanita (*Chamaebatia foliolosa*), and California black oak (*Quercus kelloggii*). There is a well developed organic horizon, approximately 20 cm in depth. The climate is also Mediterranean, with sporadic snowfall between

October and March. The soil is the Musick soil series, of the subgroup Ultic Haploxeralfs, and is also formed from weathered granodioritic parent material (Dahlgren et al. 1997).

Field methods

In December 1996, we took 150 soil cores from 1.5 by 1.5 meter quadrats at the corners and center of a 10 m^2 grid in each system (30 cores from each quadrat). A grid size of 10 m^2 was determined to be representative based on an assessment of the site microbial spatial variability (using semivariogram analysis of direct microscopic counts and substrate utilization tests; see Balser 2000). Cores of PVC tubing, 15 cm tall with a 5 cm inner diameter, were driven to 10 cm depth in the mineral soil. Half of the cores were transplanted to the other climate/ecosystem (transplant treatment), and the remaining half were replaced in their native environment as a disturbed control treatment. Intact soil within the PVC cores was capped at both ends with 1 mm mesh to allow water equilibration. Cores were placed beneath the litter layer to maintain microclimatic conditions. At time zero, 12 and 27 months, we randomly collected three replicate cores from the control and transplant treatments in the five quadrats of both ecosystems and brought them to the lab for analysis. At 27 months we also took fresh soil, using the same methods and cores, in order to assess the impact of coring vs. transplanting on total carbon and nitrogen contents.

Lab methods

Soil processes and characteristics

Within 6 h of collection, the three replicate intact cores from each quadrat were placed together into 1.89 l sealed Mason jars fitted with septa, and incubated at 25 °C for 36 h. Headspace gas (5 ml samples) was analyzed for CO_2 by thermal conductivity detector and N_2O by electron capture detector using a model 8610 gas chromatograph (SRI Instruments, Torrance, CA). Soil metabolic quotient (CO_2 production per unit biomass, 'qCO_2') was calculated from these data. The metabolic quotient is generally considered to be an indicator of community physiological stress, though it can and will vary with substrate (Anderson and Domsch 1993; Anderson 1994; Wardle and Ghani 1995). In this study, we used CO_2concentration, divided by $nmol_{lipid}$.

Following headspace gas measurements, the soil from each set of three cores was combined for a total of five site replicates. Soil was homogenized by thorough mixing; coarse roots and fragments greater than 4 mm were removed. Samples were stored at 4 °C until analysis. Composite ecosystem replicates were subsampled for the remaining process and community characterization assays. With the exception of lipid analysis, all assays were complete within 2 days. Samples for lipid analysis were frozen within 48 h of

collection, and preserved by lyophilization. Gravimetric soil water content was determined after 3 days at 105 °C. Soil results are reported per gram dry weight soil.

Total organic carbon and nitrogen of whole soils was measured by combustion to CO_2 and N_2O with a Thermo-Jarrell CN analyzer. Labile organic carbon was determined by extraction with 0.5 M K_2SO_4 by shaking on a platform shaker for 30 min (1:5 soil:extractant ratio). Following filtration with Whatman No. 41, the extractant was analyzed for organic C by acid dichromate oxidation. Potassium sulfate extractable organic carbon is used here as an index of biologically available soil carbon. Soil NH_4^+ and NO_3^- concentrations were determined by extracting 20 g, field moist subsamples from each of five ecosystem replicates with 100 ml of 2M KCl (Mulvaney, 1996).

To determine gross rates of N-mineralization and NH_4^+-consumption by ^{15}N-pool dilution, 1 ml of a solution containing ^{15}N-enriched $(NH_4)_2SO_4$ was added to 40 g subsamples of each of the treatment replicates of field moist soil in 0.24 l jars (8 oz. jelly jars). Sufficient ^{15}N (99 atom percent) was added to bring the soil NH_4^+ pools to ~20 atom percent ^{15}N. Background N concentrations were 3.2 mg kg^{-1} NH_4^+, and 4.4 mg kg^{-1} NO_3^-. After thorough mixing, 20 g subsamples were extracted for time zero measurements. A second set of subsamples were extracted after 24 h incubation at 25 °C. Sample preparation, isotope analysis and gross rate calculations have been previously described (Hart et al. 1994; Herman et al. 1995).

To determine the maximum potential rate of ammonium oxidation, the nitrification potential assay described in Hart et al. (1994) was used. Samples taken at 0, 24 and 48 h were analyzed for NO_3^- concentration on the Lachat flow-injection analyzer.

Our process measurements were primarily laboratory based. This was purposeful rather than incidental on our part. We specifically wanted the uniformity of the lab conditions. Our goal with the research was not to mimic field conditions, nor to predict field process rates. Much like a 'potential assay', because measurement conditions were consistent across all sampling times, we can more safely assume that differences we see are due to real differences in the microbial community, rather than responses to the field environment. In addition, we assessed microbial response over a relatively long time scale (2 years), while the scale of lab our measurements was short (2 days). Again, this allows us to more safely assume that differences are due to real differences in the microbial community.

Microbial community characterization

Individual fatty acids are found in the membranes of nearly all microorganisms, but because the relative amount of each fatty acid varies among organism groups, lipid profiles are useful in quantifying presence and relative abundance of gram−, gram+, actinobacteria, and fungi in soil communities (Federle 1986; Ratledge and Wilkinson 1988; Wilkinson 1988; Vestal and

White 1989; Ringelberg et al. 1997; Zogg et al. 1997). In this study, we used phospholipid fatty acid profiles (PLFA) to assess coarse-scale changes in microbial community composition. We extracted, purified and identified PLFAs from microbial cell membranes in lyophilized whole-soil using a modified Bligh and Dyer (1959) technique, following the method of Bossio and Scow (1998), with some modifications as per Ringelberg et al. (1997). We added 18:0 (octadecanoic methyl ester) and 10:0 (decanoic methyl ester) as internal standards and analyzed samples using a Hewlett Packard 6890 Gas Chromatograph with a 25 m$\times$0.2 mm$\times$0.33 μm Ultra 2 (5%-phenyl)-methylpolysiloxane column (Hewlett Packard, CA).

Community substrate utilization profiles were generated using BiOLOG^{TM} Gram-negative microtiter plates (BiOLOG Inc., CA), as described in Garland and Mills (1991) and Balser et al. (2002a), to provide an activity-related community characterization. We dispersed 20 g samples of field moist soil, and inoculated plates using an 8-channel micropipetter set to 150 μl, following a 1 h shake in 200 ml 0.7% m/m NaCl solution and serial dilution to 10^{-3}. Cell concentrations were determined by direct counts of the initial 1:10 dilution using acridine orange stain and epifluorescence microscopy. All plates were incubated at 28 °C, and read on a BiOLOG^{TM} Microplate Reader (590 nm filter) approximately every 12 h for 72–100 h.

Data handling and statistical analyses

Fatty acid peak areas were converted to mole-percent of total fatty acids using internal standards. In all subsequent analyses we used only fatty acids that were identifiable and present at 0.5 mole percent or higher. We included 10 unidentified fatty acids that were either present in very high abundance in one or more treatments, or that were present in all treatments. Mole percent values sum to a constant value (100%), and thus are not normally distributed. Taking the arcsine of the square root of each fatty acid mole fraction normalizes the data (Zar 1984). Transformed values were used for subsequent multivariate analyses, and for variable generation as described below. We used the number and abundance (non-transformed) of fatty acids to calculate fatty acid richness and diversity (Simpson's index) (Begon et al. 1986). The sum of abundance (non-transformed data) of all fatty acids is an index of microbial biomass (White et al. 1979; Zelles et al. 1992; Hill et al. 1993).

Substrate utilization profiles were analyzed by subtracting color development in the control well (due to utilization of background dissolved organic carbon and turbidity) from absorbance readings in all other wells. Negative values were set to zero. We chose a time point to analyze for each plate based on an average well color development (AWCD) of between 0.75 and 1.0 absorbance units (Garland 1996) and normalized individual well absorbance by total plate color to account for differences in inoculation density between samples. These data were used for factor analysis (principal components) to generate a microbial community summary variable based on a functional attribute (substrate utilization).

To gain the maximum 'information' from the multivariate data sets generated by the PLFA and substrate utilization assays (Selvin 1995), we used unrotated principal components as summary variables describing the microbial community. Used as an exploratory analysis technique, principal components analysis (PCA) is an unbiased data transformation that generates univariate canonical summary variables (Selvin 1995). We used one way ANOVA followed by Tukey's HSD test to assess the differences between treatments (transplant and disturbed control in two ecosystems). Soil 'treatment' was used as the independent variable, and each microbial community variable was dependent. We also assessed the site and treatment interactions (none were significant). We used multivariate linear regression to look at the relationship between microbial community and soil processes, and we partitioned the variance in R^2 (as detailed in next paragraph) in order to quantify the relative importance of each independent ('X') variable in the linear model. Data from each individual sample on each sampling date (initial, 12, and 27 months) were included in each regression analysis. Our goal in combining data across sites and times was to examine trends that emerged above variation due to treatment or time. For all analyses we used JMP statistical software (SAS Inc).

Collinearity of independent variables in a multiple regression can lead to spurious associations by obscuring their relationship with dependent variables. Partial correlation coefficients overcome this problem by allowing for the examination of the relationship between dependent and a given independent variable, while holding the effects of all other independent variables constant. Therefore, we assessed the degree to which independent variables (soil properties and microbial summary variables) account for unique variation in soil processes using partial Pearson product-moment correlations. The importance of each X variable is thus obtained by partitioning the variance in the R^2 value for the regression. The resulting percentage of the R^2 value for each X variable in the regression is *independent* of the other X variables, indicating the importance of each X in explaining variance in the regression model.

Results

The study began in December 1996 and ended in February 1999. At each of the times sampled, the grassland soil was warmer than the conifer soil (Table 1). Because of differences in its water holding capacity/water characteristic curve, the conifer soil tended to be wetter in either climate throughout the study (Table 1). Total soil C, N, and K_2SO_4-extractable C were determined at 27 months in freshly sampled soils to compare to soils that had been in place in cores and transplanted in cores. Total soil C, N, and labile C were indistinguishable by treatment within soil type (one-way ANOVA; $p > 0.3$, grassland soil, $p > 0.4$, conifer soil).

Table 1. Summary of climate and soil properties at study sites

	Annual grassland	Mixed conifer
Elevation (m)[a]	470	1240
Mean annual air temperature (°C)	17.8[a]	8.9[b]
Mean annual precipitation (cm)[a]	31	~95
Soil properties (0–15 cm)		
Soil series	Fallbrook	Musick
Bulk density (Mg m^{-3})[d]	1.4	0.98
pH[c]	5.5	5.3
%clay	10[a]	15–22[a,b]
WHC (field capacity) (kg kg^{-1})[c]	0.20	0.38
Microbial biomass[c] ($mMol_{lipid}$ kg_{soil}^{-1})	0.60	0.29
Fungal: bacterial lipid biomass ratio[c]	2.0	3.1
Soil carbon and nitrogen after 27 months transplant, 0–10 cm depth: undisturbed/in-place/transplanted; values for a given soil are not significantly different		
	Annual grassland	Mixed conifer
%C[c]	1.0/1.2/0.9	5.7/4.0/4.3
%N[c]	0.10/0.12/0.09	0.22/0.18/0.20
C:N[c]	9.8/10.1/10.2	25.8/23.0/21.9
K_2SO_4-Labile C[c]	0.10/0.14/0.11	0.22/0.15/0.14
Soil temperature at 10 cm depth during transplant (°C)		
Dec. '96	8.6	2.7
Dec. '97	17	2.0
Feb. '99	7.7	2.7
Water content (kg_{water} kg_{soil}^{-1}) during transplant (data shown are: in-place {transplanted})		
Dec. '96	0.19{0.19}	0.36{0.36}
Dec. '97	0.17{0.13}	0.15{0.29}
Feb. '99	0.18{0.16}	0.26{0.32}

[a]From Trumbore et al. (1996).
[b]From Dahlgren et al. (1997).
[c]From Balser (2000).
[d] Wang, Y. and R. Amundson, University of California, Berkeley, unpublished data.

Microbial community response to reciprocal transplant

The primary influence of the transplant on microbial community composition and function was intended to be a change in climate. Impacts of the change in climate (and location) would be apparent as separation between transplant and control bars in Figures 1–3. Controls and transplanted treatments for soils from both ecosystems generally behaved similarly, decreasing or increasing together during the course of the study Figures 1–3.

Fatty acid markers and microbial community indices generally varied over time (often increasing or decreasing statistically significantly). In only two cases did the biomarkers show community change in response to transplant; grassland monounsaturated fatty acids declined with transplant at 12 months and grassland fungi increased with transplant at 27 months (Figure 1). The increase in the relative importance of fungi in the microbial community is

however notable. Bacterial community composition in grassland soil (as assessed by PLFA guilds) appeared to be resistant to change in water and temperature regime.

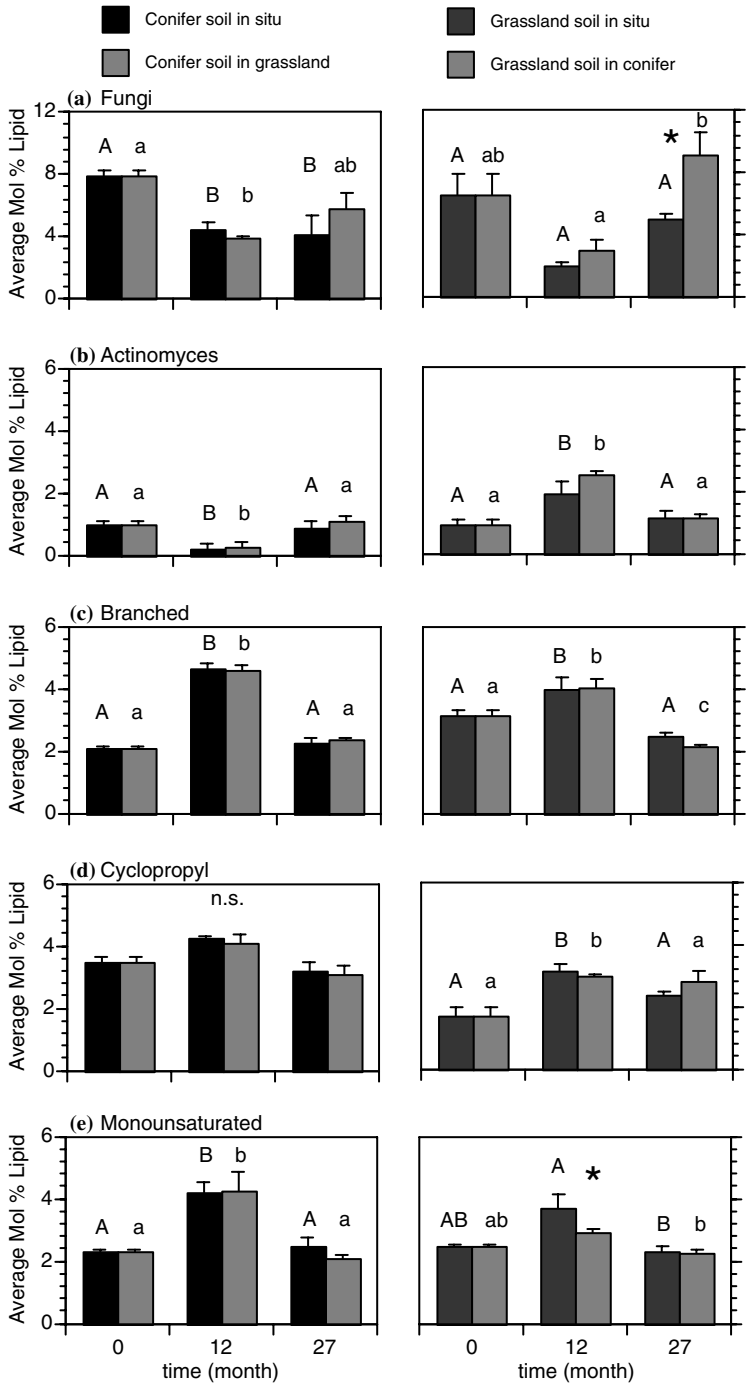
Another possible influence on microbial community response could be the effect of coring the soil. To assess this disturbance effect, we took cores from untreated soil at 27 months in order to assess the impact of coring. We found no changes in total carbon or nitrogen in cored vs. fresh soil (Table 1). In both soil types, the abundance of the fungal biomarker was lower in cored rather vs. fresh soil; possibly as a result of hyphal excision by coring. However, the inherent differences in biomarkers (community structure) between conifer and grassland soils were larger than the coring effect.

Relationship between microbial community and soil process rates

We used multiple regression analysis to assess the relationship between environmental and microbial community variables and soil process variables. We tested for interactions by site or time, and found no significant results. Neither did we find evidence of a functionally two point regression (as would be indicated if data from the regression analysis clustered according to site). We evaluated microbial biomass values and lipid diversity indices as well as first principal component values from substrate utilization and PLFA in regression models. Neither microbial biomass nor diversity indices were related to soil process rates (data not shown). In contrast, some principal component values were significantly related to soil processes (Table 2).

Multiple regression analyses with soil water, soil temperature (taken at the time of core harvest), and two microbial community summary variables (first principal component from substrate utilization and PLFA analyses: SU_PC1 and PLFA_PC1) indicate that there are relationships between microbial community and soil processes independent of environment (Table 2). Only gross ammonium immobilization was not significantly related to either environment (soil T and water) or microbial community (SU_PC1 and PLFA_PC1). Significant relationships were found between field soil T and both CO_2 production and soil NH_4 concentration. In four of the six significant relationships, the two microbial community variables accounted for the largest percent of the variance in the soil process variable (had the largest % of R^2 ; Table 2). In fact, for nitrification potential and nitrate pool ($[NO_3^-]$), the BiOLOG-substrate utilization and PLFA variables together accounted for greater than 90% of the variance explained by the multivariate model.

To further explore the relationship between microbial community composition and soil processes, we analyzed those soil processes that were significantly related to microbial community composition from Table 2 (nitrous oxide flux, nitrification potential, nitrate pool size, and gross ammonium mineralization). We quantified the relationship between PLFA 'guilds' and


Conifer soil in situ
Conifer soil in grassland
Grassland soil in situ
Grassland soil in conifer
(a) Fungi
(b) Actinomyces
(c) Branched
(d) Cyclopropyl
(e) Monounsaturated
Average Mol % Lipid
n.s.
time (month)
0
12
27

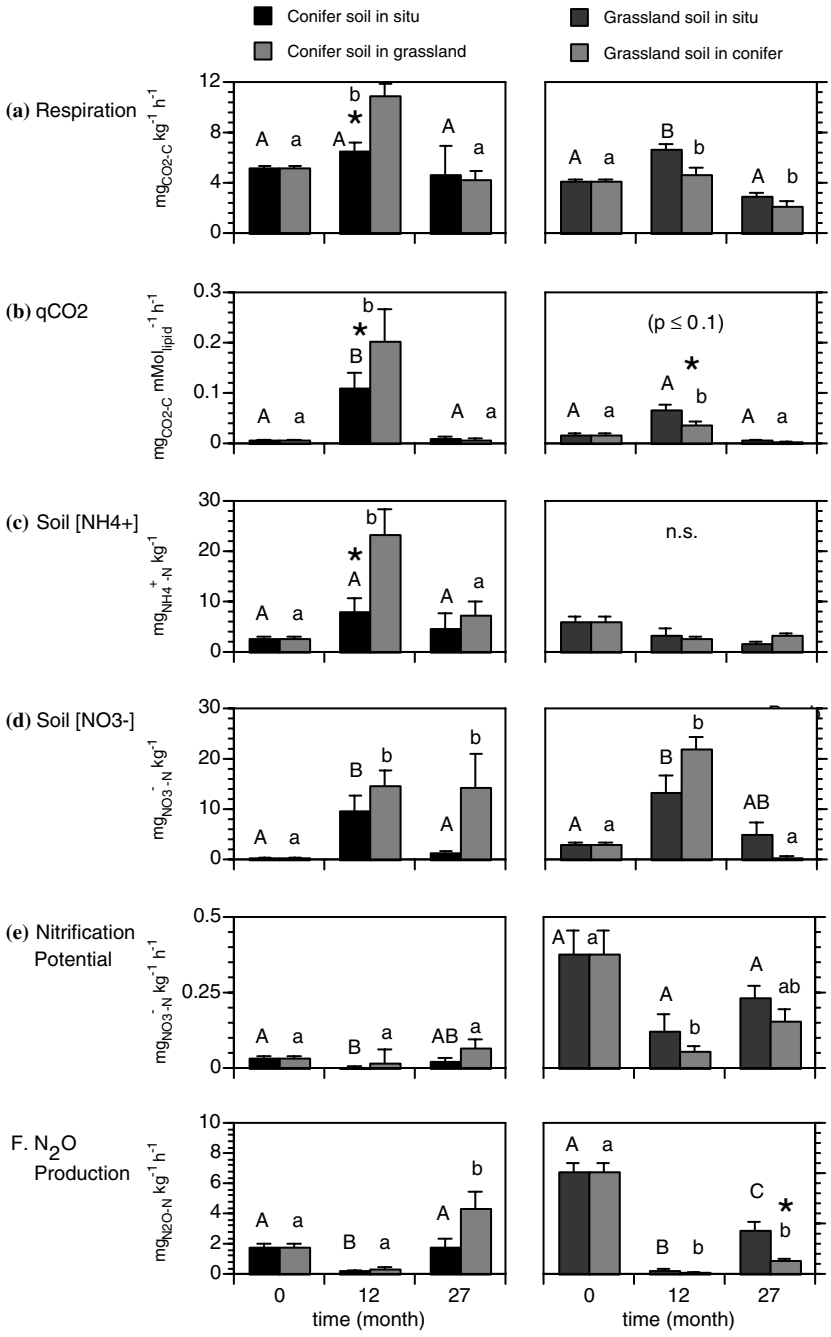
each soil process. For three of the processes, there was a dominant guild that accounted for greater than 70% of the variance explained by the model (Table 3). Cyclopropyl fatty acids, generally indicative of gram-negative bacteria, were specifically related to nitrification potential and N_2O flux. Nitrate concentration ($[NO_3^-]$) was related to branched fatty acids indicative of gram-positive bacteria. Gross N mineralization broadly related to several guilds with the fungal marker accounting for the highest percentage of the R^2 value (Table 3).

Effect of soil transplant on soil process rates and characteristics

The largest response to transplant was observed in conifer soil transplanted to the grassland climate. Carbon dioxide flux and $[NH_4^+]$ at 12 months were significantly greater in transplanted conifer soil than conifer in-place soil (Figure 2). In addition, the metabolic quotient (qCO_2), was significantly different at 12 months for control vs. transplanted soil cores (Figure 2). A high qCO_2 generally indicates low metabolic efficiency (high utilization of carbon per unit biomass produced), indicative of a community that is stressed. In this study, the qCO_2 was highest in conifer soil transplanted to the grassland ecosystem (Figure 2).

Transplant significantly decreased N_2O production in grassland soil moved to the conifer site after 27 months and significantly increased CO_2 production and soil NH_4 concentration in conifer soil transplanted to grassland at 12 months. There were no significant effects of transplant on gross mineralization/consumption (data not shown), nitrification potential, or soil solution NO_3^- concentration (Figure 2). In addition, there was no significant change in soil total C, N or labile C after 27 months in transplanted soils (Table 1).

Figure 1. Relative abundance of fatty acids in organism guilds during 2 year soil transplant experiment. The abundance of fatty acid biomarkers in each of five guilds (fungi, actinomycetes, branched, cyclopropyl and monounsaturated) were summed and normalized by the number of members in the guild to obtain relative abundance and to allow comparison between guilds. Error bars denote one standard error of the mean ($n = 5$). Solid bars represent in-place soil and hatched bars represent transplanted soil. Letters above the bars are a comparison of either in-place soil across the three times (capital letters), or transplanted soil compared across the times (lowercase letters). Bars with either the same capital or lower case letter are not significantly different by Tukey's HSD test ($p < 0.05$). An asterisk (*) above two bars indicates a significant difference between transplanted and in-place soil ($p < 0.05$ unless noted). (a) Fungal abundance; (b) Actinomycetes; (c) Branched; (d) Cyclopropyl; (e) Monounsaturated. At the 12 months sampling, Actinomycetes and Cyclopropyl abundance differed between conifer and grassland soils ($p < 0.05$ by ANOVA).


Conifer soil in situ
Conifer soil in grassland
Grassland soil in situ
Grassland soil in conifer
(a) Respiration
(b) qCO2
(c) Soil [NH4+]
(d) Soil [NO3-]
(e) Nitrification Potential
F. N2O Production
(p ≤ 0.1)
n.s.
time (month)
time (month)

Discussion

Relationships among soil processes, environment and microbial community

In past ecosystem studies, microbial communities have commonly been represented by biomass or diversity indices. We compared the relationship of soil process rates to biomass, a diversity index based on lipid profiles, a diversity index based on substrate utilization, and the results of a multivariate reduction method (PCA) as community parameters. Despite limitations imposed by the size and scope of this study, our data indicate two interesting points. First, in spite of slight changes over time (temporal dynamics) during the study (Figure 3), microbial diversity and biomass indices were not related to soil process variables in any statistical model evaluated. This is an important point to note: soil transplantation over 2 years in both cases did not affect microbial biomass or diversity. This is in marked opposition to what might have been expected, based on our conceptual models of soil microbial dynamics. Second, while biomass and lipid diversity were unrelated to soil processes, microbial community structure (as indicated by the first principal component of PLFA) *was* significantly related. The use of ordination (PCA) to generate microbial summary variables appears to be a valuable approach for including microbiological information in statistical and mechanistic models of soil functioning. Broad-based indices such as biomass or lipid diversity may not be sensitive enough to reflect changes in soil process rates.

Microbial community characteristics (represented by PC1 from lipids and substrate utilization) appeared to be related to soil processes independent of environmental variables (soil temperature and water) (Table 2). Gross N mineralization, nitrification potential, nitrous oxide flux, and NO_3^- pool size were all dominantly related to microbial community composition (PLFA PC1) rather than to field environmental variables (water, temperature). Carbon dioxide flux and NH_4^+ concentration were more strongly related to environmental variables (temperature). These data are consistent with Schimel's (1995) ideas about broad vs. narrow physiological groupings. When organisms fall into a relatively narrow taxonomic group (such as the nitrifying bacteria), then their presence, absence, or abundance may be more related to the process they

Figure 2. Soil process rates and N pool sizes during 2-year transplant. Rates are laboratory values. Error bars denote one standard error of the mean ($n = 5$). Letters above the bars are a comparison of either in-place soil across the three times (capital letters), or transplanted soil compared across the times (lowercase letters). Bars with either the same capital or lower case letter are not significantly different by Tukey's HSD test ($p < 0.05$). An asterisk (*) above two bars indicates a significant difference between transplanted and in-place soil ($p < 0.05$ unless noted). (a) CO_2 production ($mg_{CO_2-C}\ kg_{soil}^{-1}\ h^{-1}$); (b) $qCO_2(mmol_{lipid}\ kg_{soil}^{-1}\ h^{-1})$; (c) Soil $[NH_4^+]$ ($mg_{NH_4-N}\ kg_{soil}^{-1}$); (d) Soil $[NO_3^-]$ ($mg_{NO_3-N}\ kg_{soil}^{-1}$); (e) Nitrification potential ($mg_{NO_3-N}\ kg_{soil}^{-1}\ h^{-1}$); (f) N_2O production ($mg_{N_2O-N}\ kg_{soil}^{-1}\ h^{-1}$).

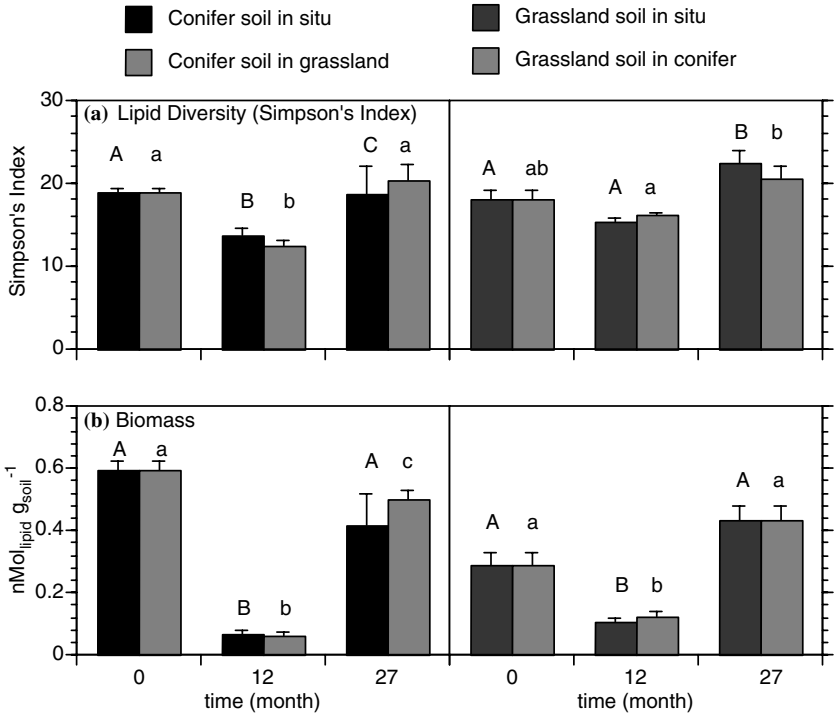


Figure 3. Microbial lipid-diversity (Simpson's Index) (a), and biomass ($mMol_{lipid}$ kg_{soil}^{1}) (b) during 2-year transplant. Error bars denote one standard error of the mean ($n = 5$). Letters above the bars are a comparison of either in-place soil across the three times (capital letters), or transplanted soil compared across the times (lowercase letters). Bars with either the same capital or lower case letter are not significantly different by Tukey's HSD test ($p < 0.05$). An asterisk (*) above two bars indicates a significant difference between transplanted and in-place soil ($p < 0.05$ unless noted).

carry out, than are organisms that perform a function covered by a broad spectrum of organisms (such as CO_2 flux).

Specific microbial community components and processes

One dominant PLFA 'guild' explained greater than 70% of the variance in the relationship between PLFA guilds and three of the soil processes (Table 3). In contrast, gross N mineralization (a process performed by most soil heterotrophs), was more broadly related to fatty acid guilds (Table 3). The cyclopropyl fatty acids (generally indicative of gram-negative bacteria) were most strongly related nitrification potential and N_2O flux. Cyclopropyl fatty acids may be linked to these two processes via two mechanisms. First, autotrophic nitrification is carried out by gram-negative bacteria that are collectively in the

Table 2. Effects of microbial community and environment on soil processes: multiple regression analysis and partitioning the variance in R^2

Y variable	X variable	R^2	$p > F$	% of R^2
N_2O		0.476	< .0001	
	T			13.8
	Water			13.2
	SU PC1			1.7
	PLFA PC1			71.3
Nitrification		0.141	0.0394	
Potential	T			5.2
	Water			0.26
	SU PC1			5.3
	PLFA PC1			89.3
$[NO_3^-]$		0.459	< .0001	
	T			0.05
	Water			1.5
	SU PC1			39.5
	PLFA PC1			59.0
CO_2		0.565	< .0001	
	T			64.7
	Water			1.4
	SU PC1			25.5
	PLFA PC1			8.5
$[NH_4^+]$		0.297	0.0001	
	T			54.2
	Water			15.7
	SU PC1			14.4
	PLFA PC1			15.7
Gross NH_4^+		0.188	0.0081	
Mineralization	T			14.8
	Water			13.8
	SU PC1			4.4
	PLFA PC1			67.1
Gross NH_4^+			Not significant	
Consumption	T			
	Water			
	SU PC1			
	PLFA PC1			

All sampling times combined. PC1SU and PC1PLFA are the first principal components of substrate utilization and PLFA respectively. The relative importance of each 'X' variable in the regression is indicated by the % of R^2 for which it accounts. T and W are the soil temperature and water content when the cores were harvested.

family Nitrobacteriaceae, and nitrous oxide is often a by-product of nitrification (Firestone and Davidson 1989). Second, cyclopropyl fatty acids can also indicate anaerobic bacteria (Bossio and Scow 1998). Denitrification is an anaerobic process generating N_2O. It is interesting that these two processes can potentially be linked to the relative abundance of gram-negative bacteria using fatty acid analysis. The ability to link the structure and associated function of microbial communities is highly desired.

Table 3. Relationship between fatty acid guilds and soil processes

Y variable	X variables	R^2	$p > F$	% of R^2
N_2O production		0.45	< .0001	
	Actinomycete			9.8
	Branched			2.8
	Cyclopropyl			71.1
	Fungi			9.9
	Monounsaturated			6.4
Nitrification Potential		0.33	< .0001	
	Actinomycete			0.27
	Branched			6.7
	Cyclopropyl			87.9
	Fungi			2.8
	Monounsaturated			2.4
Gross N Mineralization		0.20	0.0115	
	Actinomycete			2.3
	Branched			13.5
	Cyclopropyl			29.1
	Fungi			35.5
	Monounsaturated			19.5
$[NO_3^-]$		0.30	0.0003	
	Actinomycete			0.50
	Branched			76.3
	Cyclopropyl			2.5
	Fungi			13.7
	Monounsaturated			7.0

Only soil processes that are significantly related to microbial community composition (PC1PLFA) are included here. See text for explanation of partitioning variance in R^2.

The solution concentration of nitrate, the product of nitrification, was positively related to branched fatty acids indicative of gram-positive bacteria. Gram-positive bacteria could be related to nitrate pool size through two possible mechanisms: as nitrate consumers (Stark and Hart 1997) or through heterotrophic nitrification. Heterotrophic nitrification has been shown to be dominantly carried out by fungi and gram-positive bacteria (Schlegel 1993; Myrold 1998). It is commonly thought to occur in forest soils (Pedersen et al. 1999), but has been found to occur in a wide range of soils (Barraclough and Puri 1995).

Analysis of fatty acid profiles and soil process rates appears to confirm the importance of focusing on the link between microbial community composition and phylogenetically clustered soil processes. In addition, however, we found evidence that microbial community composition and physiology can also influence processes that are performed by many soil organisms (e.g. gross N mineralization) independent of a change in environment.

Importance of antecedent microbial community in response to environmental change

In this study, qCO_2 was highest for the conifer soil moved to the grassland site after 12 months, suggesting that the conifer microbial community was stressed by the move to a new climate. The grassland community was less stressed by its move to the conifer climate. The reason for the difference may have to do with the native climate to which the community was adapted. The California annual grassland climate has a wider annual range in temperature and water potential than mid-elevation mixed-conifer forests in the southern Sierra Nevada (Schoenherr 1992; Trumbore et al. 1996; Dahlgren et al. 1997). At these sites, the grassland reached 47 °C at ten cm soil depth in July, and reached a low of 8 °C in December (Balser, *unpublished data*). A move to the conifer climate did not exceed the 'normal' range of the grassland community. In contrast, the transplanted conifer soil community experienced a much hotter, drier summer than that to which it was adapted. As a result the conifer community may have been more affected by the transplant than the grassland microbial community. This impact of environmental stress was indicated by more by activity (qCO_2), than by a change in microbial community structure, as we saw no significant differences in biomass or lipid diversity (Figure 3). It does not necessarily follow that a change in microbial activity must be accompanied by a change in taxonomic structure, nor vice versa. Nor were the differences in qCO2 likely due to differences in carbon substrate availability. The transplanted cores were kept intact, maintaining the carbon 'environment' native to each microbial community (grassland or conifer-based). In addition, there were no significant differences in total or labile carbon within a soil type among fresh soil, transplanted soil, or soil incubated in place (Table 1).

This effect of antecedent conditions may have implications for models predicting ecosystem functioning in response to environmental changes such as change in land cover, altered tillage regimes, or changes in the seasonality and frequency of precipitation. Each of these environmental changes has the potential to stress a microbial community that is in 'equilibrium' with its native climate. In this study we saw that microbial community response to disturbance was specific to grassland and conifer ecosystems. The conifer community was more sensitive to change. This sensitivity may have had an impact on nitrogen cycling in the conifer soil. Thus predicting nitrogen cycling in response to environmental changes may be not only a function of abiotic factors, but also a function of the preexisting adaptation of the microbial community and its physiological plasticity.

Timescale of microbial community response to transplant

Both microbial biomass and microbial community composition remained surprisingly constant in response to transplant over a 2-year period (Figure 1).

Despite a small shift in fungal abundance in grassland soil at 27 months, and despite occasionally dogmatic beliefs to the contrary, our data do not indicate a rapid equilibration of microbial community composition to changing climatic factors. The time-scale over which microbial communities in natural soils respond to disturbance has not been well quantified (Balser et al. 2002b). We may need to re-evaluate our assumptions (often embedded in models of ecosystem functioning) about the immediate equilibration of microbial communities with changing climate. Our data indicate the possibility that changes in a soil microbial community could influence nitrogen availability, and thus perhaps ecosystem scale events such as plant competitive balance, successional trajectory, or seedling establishment, even on a relatively short time-scale. The speed of microbial community adaptation or shift could be a factor in the movement of future life-zone boundaries as plant communities adjust to changes in global climate (Walker and Steffan 1999).

Conclusions

Regional to global models of soil process or ecosystem functioning often treat microbial community composition as a static variable, or as one in permanent equilibrium with abiotic drivers. Such models primarily use environmental variables (such as temperature, or water content) to drive process rates. The data we present in this paper, however, suggest that knowledge of the response of microbial communities to climate change and disturbance may be of value in predicting the response of ecosystems to future changes.

In this study, we used microbial community assays in conjunction with laboratory assays of soil processes to assess the relative importance of microbial community composition and environmental factors in constraining soil process rates. The study was based on a relatively small number of samples, but our primary goal was not to extrapolate to the field scale. Instead the study was designed to address whether microbial community composition might constrain soil process rates and what microbial parameters might be best in assessing the link between community composition and soil process.

We found no significant relationship between microbial biomass, or lipid diversity, and soil process rates. We also found that the first principal component was a good summary variable to represent the microbial community, and could be related to soil processes. Our data indicated that microbial community characteristics may mediate or constrain soil process rates independent of environmental changes. More specifically, we found a relationship between specific components of the microbial community and those soil processes that are more tightly linked to microbial phylogeny. In addition, we found that microbial community composition may not equilibrate to an environmental change as readily or as rapidly as we have often assumed. If microbial communities evolve or shift slowly in response to environmental change, and particularly if their response potentially constrains soil process

rates, then it may be advantageous to include parameters quantifying microbial community responses in our conceptual and mechanistic models of ecosystem functioning.

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