Microbial Ecology

Relationships Between Microbial Community Structure and Soil Processes Under Elevated Atmospheric Carbon Dioxide

David A. Lipson[1], Michelle Blair[1], Greg Barron-Gafford[2], Kathrine Grieve[2] and Ramesh Murthy[2]

(1) Department of Biology, San Diego State University, San Diego, CA 92182-4614, USA
(2) Biosphere2 Laboratory, Oracle, AZ 85623, USA

Received: 28 August 2005 / Accepted: 2 September 2005 / Online publication: 6 April 2006

Abstract

There is little current understanding of the relationship between soil microbial community composition and soil processes rates, nor of the effect climate change and elevated CO_2 will have on microbial communities and their functioning. Using the eastern cottonwood (*Populus deltoides*) plantation at the Biosphere 2 Laboratory, we studied the relationships between microbial community structure and process rates, and the effects of elevated atmospheric CO_2 on microbial biomass, activity, and community structure. Soils were sampled from three treatments (400, 800, and 1200 ppm CO_2), a variety of microbial biomass and activity parameters were measured, and the bacterial community was described by 16S rRNA libraries. Glucose substrate-induced respiration (SIR) was significantly higher in the 1200 ppm CO_2 treatment. There were also a variety of complex, nonlinear responses to elevated CO_2. There was no consistent effect of elevated CO_2 on bacterial diversity; however, there was extensive variation in microbial community structure within the plantation. The southern ends of the 800 and 1200 ppm CO_2 bays were dominated by β-Proteobacteria, and had higher fungal biomass, whereas the other areas contained more α-Proteobacteria and Acidobacteria. A number of soil process rates, including salicylate, glutamate, and glycine substrate-induced respiration and proteolysis, were significantly related to the relative abundance of the three most frequent bacterial taxa, and to fungal biomass. Overall, variation in microbial activity was better explained by microbial community composition than by CO_2 treatment. However, the altered diversity and activity in the southern bays of the two high CO_2 treatments could indicate an interaction between CO_2 and light.

Introduction

Soil microbial communities are central in controlling how terrestrial ecosystems respond to global change, yet the ecological role of the majority of soil microbes remains undescribed. It is well established that plants generally respond to elevated CO_2 with increased root production and exudation [21, 22]. However, the resulting effects on microbial biomass, activity, and diversity have been far from predictable. Microbial biomass either decreases [6, 43], increases [50, 52], or is unaffected by elevated CO_2 [35, 39]. There is sometimes an increase in microbial activity [11, 54], and an increased reliance on recently fixed plant carbon [37, 48]. In other studies, microbial respiration and litter decomposition rates are decreased [38, 49]. Studies on the effects of increased CO_2 on soil microbial community composition have also drawn contradictory results. In many studies, elevated CO_2 has been found to have subtle or no detectable effects on microbial community composition [7, 12, 19, 53], whereas in other studies, changes have occurred [11, 28, 40].

Much of the uncertainty in how microbial communities will respond to global change stems from the general lack of understanding how genetic diversity and function are linked in soil microbial communities [46]. Whether there exist any predictable responses of soil microbial communities to elevated CO_2 that mediate effects on biogeochemistry, or whether responses are always community-specific and unpredictable remains to be discovered. The initial goals of this study were to determine the effects of elevated CO_2 on microbial community composition and function in a model agrofor-

Present address of Greg Barron-Gafford: Department of Ecology and Evilutionary Biology, University of Arizona Tucson, AZ 85721, USA
Present address of Katherine Grieve: Quantitative Ecology and Resource Management, University of Washington, Box 352182 Seattle, WA 98195, USA
Correspondence to: David A. Lipson; E-mail: dlipson@sciences.sdsu.edu

 DOI: 10.1007/s00248-006-9032-1 • Volume 51, 302–314 (2006)

Table 1. Environmental and soil characteristics of *Populus deltoides* plantation at B2L

	East (400 ppm)		*Central (800 ppm)*		*West (1200 ppm)*	
	North	*South*	*North*	*South*	*North*	*South*
Total Sand	44.5	41.0	39.9	44.0	43.9	39.4
Coarse[a]	27.8	24.0	21.3	26.5	24.2	19.9
Fine[b]	16.7	17.0	18.6	17.6	19.8	19.5
Silt	31.7	35.5	35.0	31.8	33.7	34.9
Clay	23.8	23.5	25.1	24.2	22.3	25.7
pH	7.25	7.20	7.36	7.29	7.30	7.19
OM	0.058	0.049	0.052	0.050	0.059	0.058
GWC	0.193	0.153	0.243	0.202	0.305	0.179
PAR	749.0	780.0	624.4	669.7	581.9	713.2

Soil texture (%), pH, organic matter (g OM g^{-1} soil), and gravimetric water content (g H_2O g^{-1} soil, measured in August). Photosynthetically active radiation (PAR) summed over the month of September (mol photons m^{-2}).
[a]0.25–2.0 mm.
[b]0.050–0.25 mm.

estry ecosystem, specifically testing the hypothesis that elevated CO_2 would lead to increased biomass and activity, and to altered community structure. Perhaps, most interestingly, the resulting data set also allowed us to relate variation in soil process rates to variation in microbial community structure.

Methods

Elevated CO_2 and Drought Treatments. This study was conducted in the eastern cottonwood plantation at the Biosphere 2 Laboratory (B2L) located in Oracle, AZ, USA (32.5°37.13′N, 110°47.05′W; 1200 m asl). The plantation is partitioned by a transparent polyvinylchloride (PVC) curtain into three 41 × 18 m rectangular sections, or bays, oriented from north to south. The soil, a silt loam (28% sand, 55% silt, 17% clay), is approximately 1 m deep overlaying a concrete floor [32, 45]. The design and operation of B2L are described in detail elsewhere [13, 34, 51]. Eastern cottonwoods (*Populus deltoides*) were planted in May 1998. Photosynthetically active radiation (PAR) was at least 70% of that outside the structure, although negligible UV penetrated the glass [13]. Temperatures were generally maintained at a 32/27°C day/night cycle. During winter, after leaf senescence, temperatures were lowered to a 20/12°C cycle to harden the buds and force dormancy. Daytime atmospheric CO_2 concentrations in the east, central, and west bays were maintained near 400, 800, and 1200 ppm, respectively, during each growing season since May 2000. Outside air and CO_2 from a liquid storage tank were used to maintain the set point [8, 13, 33, 51]. Soil moisture and vapor pressure deficit (VPD) were automatically maintained.

Soil Sampling. Soils used in this study were collected on two dates in 2002. The purpose of the first sampling date in August was to intensively study soil microbial processes in a limited number of spatial replicates. Six samples were collected from each bay (18 total). The samples were each collected from within 0.5 m of a tree, in a 3 × 2 grid pattern over the entire bay. Soil cores were collected to a depth of 15 cm using 5-cm-diameter

Table 2. Diversity indices calculated individually for the 6 plots, and for combinations of the plots

Plot	*Chao1*	*Unique/total*
NE	200[a]	1.00
SE	73 (69)	0.78
NC	128 (128)	0.93
SC	18 (6)	0.50
NW	264[a]	1.00
SW	27 (15)	0.63
N	677 (700)	0.91
S	142 (79)	0.53
All	411 (144)	0.68

The Chao1 estimator is given with standard deviations. "Unique/total" is the number of observed distinct sequences divided by the total number of sequences for the indicated plot.
[a]When no duplicate sequences were observed, no error term was calculated.

Table 3. 16S rRNA sequences appearing in clone libraries three or more times, closest cultured relative (if one exists) and the accession number of the closest blast match (not necessarily the closest cultured relative)

Occurrences	*Found in plots*	*Closest known relative*	*Closest BLAST*
15	NC, SC, SE, NW, SW	*Rhodopseudomonas*	AY214810
8	SC, SW	*Delftia*	AF506992
7	SC, SW	*Leptothrix*	AY212684
4	SC, SE, SW	*Burkholderia*	AF469363
4	SC, SW	*Stenotrophomonas*	AF280434
3	SE, SC	Uncultured *Acidobacterium*	AF392737

Letters E, C, and W refer to east, central, and west CO_2 treatment bays (400, 800, and 1200 ppm CO_2, respectively); N: north; S: south.

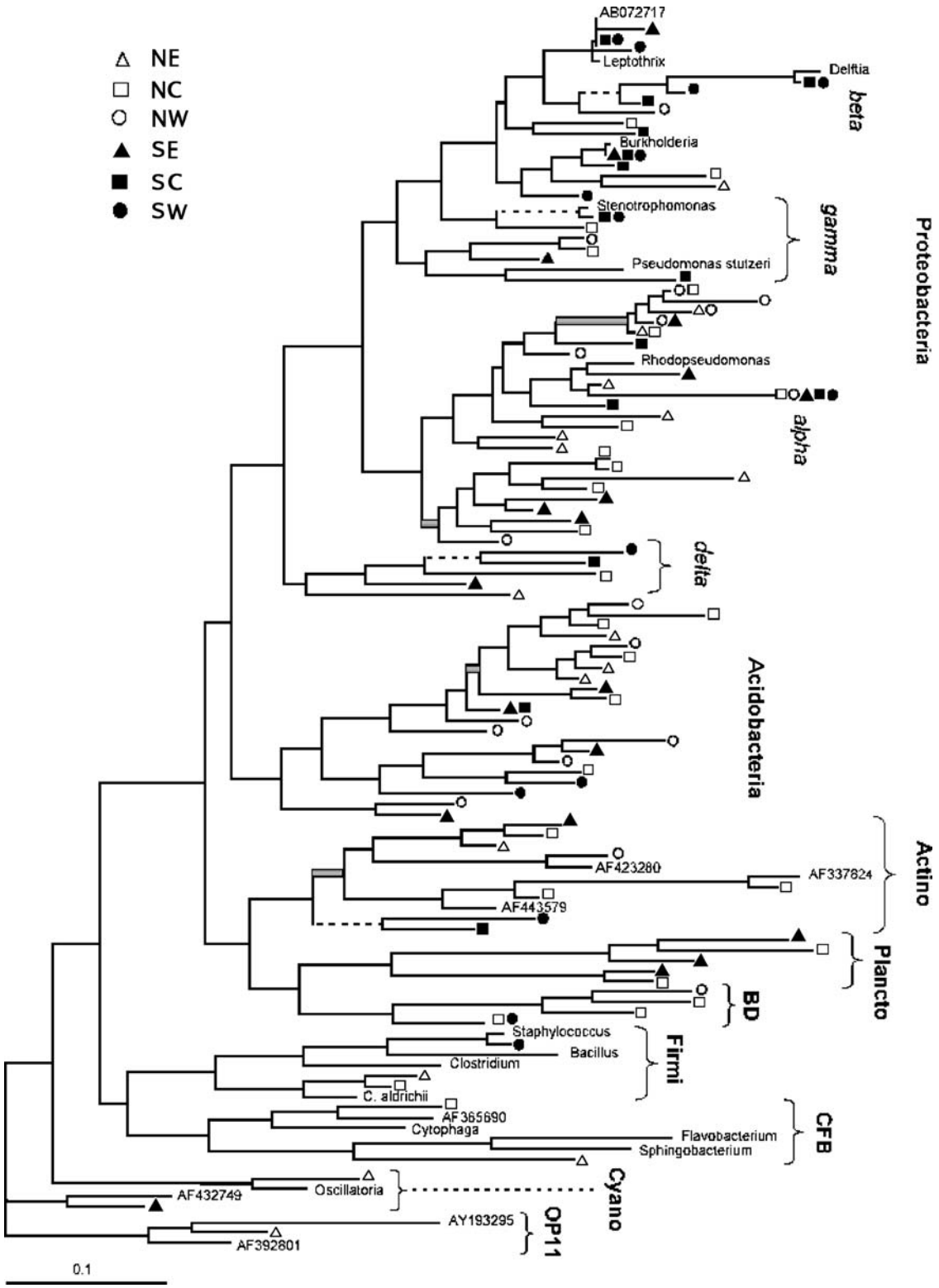

Figure 1. Maximum likelihood phylogenetic tree of 16S rRNA sequences from B2L soil. Sequences from the north (N) or south (S) sides of the east (E), central (C), and west (W) bays (400, 800, and 1200, ppm CO_2, respectively). Branches with only SC and SW sequences are indicated by *dotted lines*, branches with only NE, NC, NW, and SE sequences are indicated by *thick gray lines*. Actino: Actinobacteria; Plancto: Planctomycetes; BD: BD/*Gemmatimonas*; Firm: Firmicutes; CFB: Cytophaga–Flexibacter–Bacteroides (Bacteroidetes); Cyano: Cyanobacteria.

PVC pipe. These samples were used for measurements of microbial biomass and activity described below. High spatial variability was revealed in these initial measurements, so subsequent sampling used a more extensive approach. In October, 18 soil cores were collected from each bay (54 total) to more completely capture the spatial variability within the bays. These consisted of three samples from each of six plots within each bay. The six plots in each bay corresponded to the old agricultural plots in the initial Biosphere 2 experiment [51]. For construction of clone libraries, the October soil samples within the north or south ends of each bay were mixed before DNA extraction, resulting in a total of six samples, each representing a mixture of nine soils. Soil organic matter (OM) (loss on combustion at 500°C, 24 h) and gravimetric water content were determined for all soil samples. Soil pH was measured in a slurry of 2:1 $CaCl_2$ (10 mM)/soil. The samples used for molecular analysis of bacterial diversity were kept frozen (−80°C) until analysis. The samples used for measurements of microbial activity were kept cool (0–4°C) until analysis.

16S rRNA Clone Libraries. Soil was extracted using a modified bead beating protocol [29]. Tubes containing approximately 5 g soil samples, 2.0 g zirconia/silica beads (0.1 mm, BioSpec Products, Bartlesville, OK, USA), and 10 mL lysis buffer (TE with 0.2% SDS) were vortexed (Vortex Genie II, Fisher Scientific, Hanover Park, IL, USA) on maximum speed for 5 min. To this, 30 units of proteinase K and 10 units lysozyme (Fisher Bioreagents) were added and samples were incubated in a shaking incubator (37°C, 100 rpm) for 1 h. Standard protocols were used to purify DNA using CTAB extraction [3]. DNA was further purified using sepharose 4-D columns (Sigma, St. Louis, MO, USA) [47]. Bacterial 16S rRNA genes were amplified using universal bacterial primers f8-27 (5′-AGAGTTTGATCCTGGCTCAG-3′) and r1510 (5′-GGTTACCTTGTTACGACTT-3′) [1]. Polymerase chain reactions (PCR) were run for 32 cycles, using an annealing temperature of 56°C, with 3.0 mM $MgCl_2$, 0.2 mM of each dNTP, 1 μm of each primer, 1 g/L BSA, 50 mM Betaine, 1 unit Fisher *Taq* polymerase, and "Buffer A" supplied with the enzyme (Fisher Biosciences). PCR product was gel purified (Quiex II, Qiagen). The purified product was cloned using the TOPO TA cloning kit (Invitrogen). Clones from the six libraries were selected at random and partially sequenced on a Prism 3100 capillary electrophoresis DNA sequencer (ABI) using universal bacterial primer r536 (5′-GTATTACCGCGGCTGCTGG-3′) [1]. A total of 149 sequences were produced. Of these, 139 were of sufficient length and quality for inclusion into the phylogenetic trees. The remaining sequences were used only for BLAST searches to determine the frequency of bacterial divisions within the libraries. The unique sequences used for phylogenetic analysis were submitted to GenBank http://www.ncbi.nlm.nih.gov), and were assigned accession numbers AY632422–AY632531.

Microbial Biomass and Activity. Substrate-induced respiration (SIR) using glucose, potassium glutamate, sodium salicylate, and glycine were performed as described earlier [24]. Glucose and glutamate represent general heterotrophic microbial activity and biomass, whereas

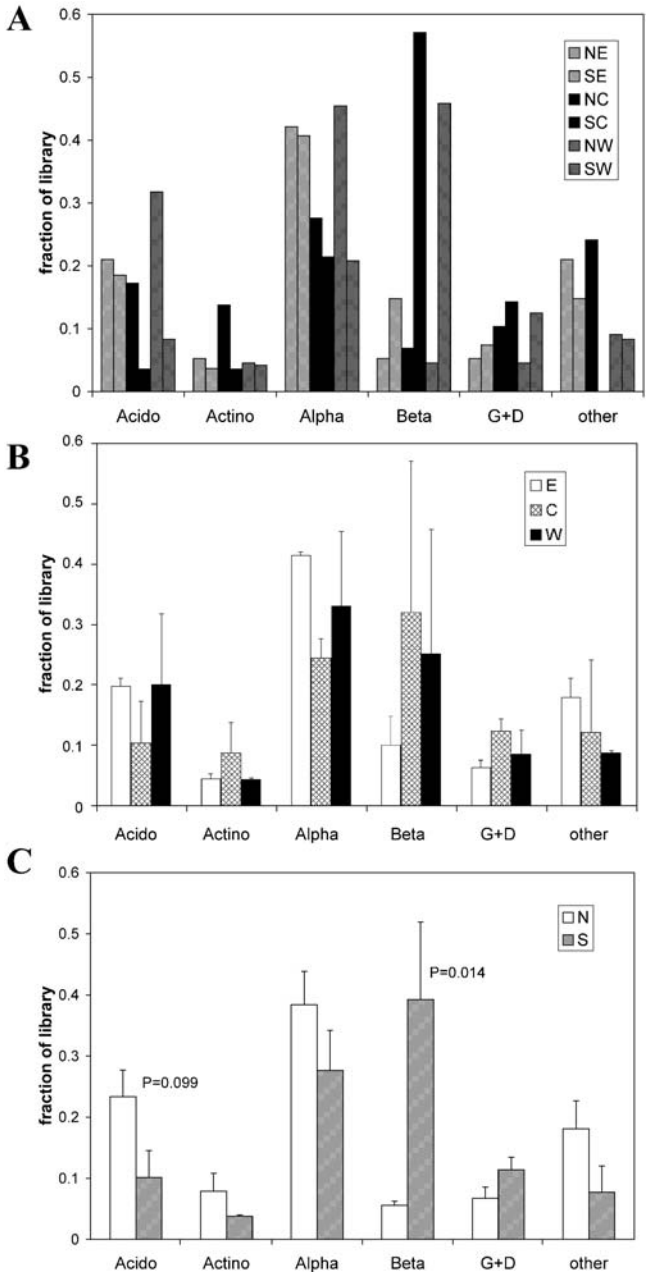

Figure 2. Frequencies of major bacterial lineages in the six bacterial clone libraries. (A) Data for individual libraries, (B) data grouped by CO_2 treatment, (C) data grouped into north and south plots. Acidobacterium (Acido), Actinobacteria (Actino), α-Proteobacteria (Alpha), β-Proteobacteria (Beta), γ- and δ-Proteobacteria (G + D).

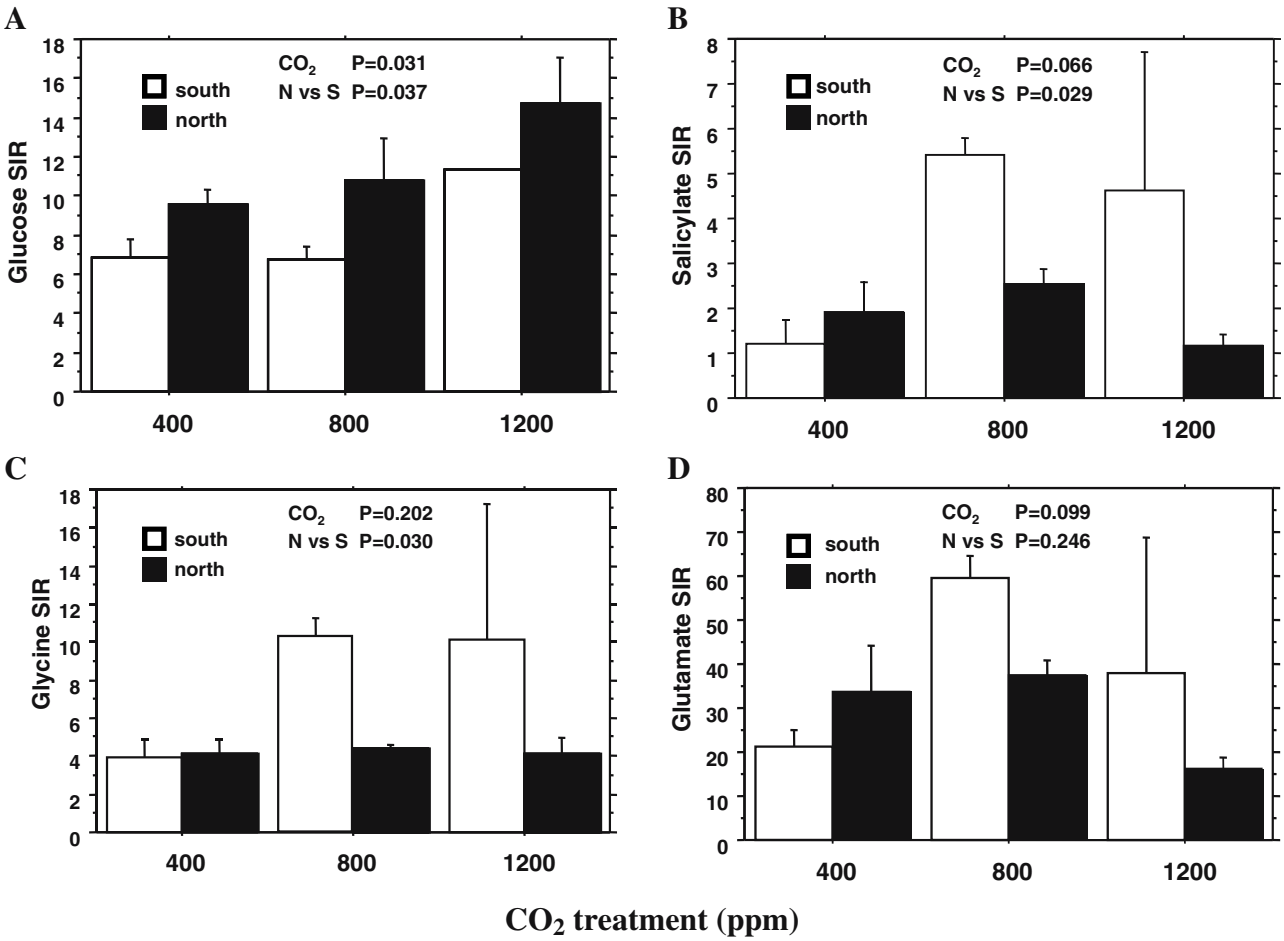

Figure 3. Substrate induced respiration (SIR, in μg C g^{-1} h^{-1}) in soils from the three CO_2 treatments (shown nominally in ppm CO_2), amended with (A) glucose, (B) salicylate, (C) glycine, and (D) glutamate. The results of the two-way ANOVA are shown for each. The interaction terms were not significant.

glycine and salicylate are degraded by smaller, more specialized functional groups. SIR was performed with soils near-optimal water content (~60% of field capacity). Fungal hyphal length was measured microscopically on samples stained with trypan blue, using a grid-intersection method [23]. The activities of extracellular enzymes in breaking down carboxy- methylcellulose (a soluble cellulose analog) and starch (amylase activity) were measured as described earlier [23]. The breakdown of Poly R478 (Sigma), a dye degraded by ligninase enzymes, was measured by the disappearance of purple color (478 nm) over time from a soil slurry buffered in 10 mM phosphate (pH 7.0) with an initial dye concentration of 1 g/L. Protease activity was assayed with casein as a substrate [24].

Phylogenetic Analysis. DNA sequences were edited using BioEdit http://www.mbio.ncsu.edu/BioEdit) [14]. Chimeric sequences were screened out using Chimera_Check http://rdp.cme.msu.edu). Representative guide sequences were selected and downloaded from GenBank http://www.ncbi.nlm.nih.gov) using BLAST searches. Sequences were initially aligned using the ClustalW algorithm within BioEdit [44], and then adjusted manually. Maximum likelihood trees were made

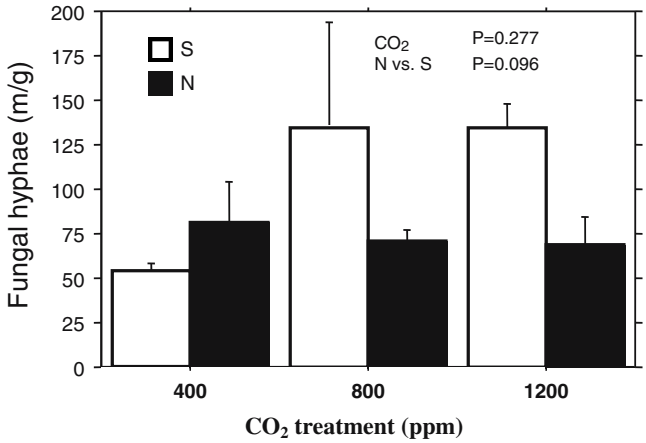

Figure 4. Fungal hyphal length in soils from the north and south plots of the three CO_2 treatments.

Table 4. Extracellular enzyme activities in north and south sides of the three CO_2 treatment bays (east, central and west)

Bay	*Plot*	*Amylase*	*Cellulase*	*Ligninase*	*Protease*
East (400 ppm)	north	103 (5.7)	55.7 (4.1)	4.81 (0.83)	47.0 (1.0)
East (400 ppm)	south	433 (250)	52.3 (15.1)	2.97 (0.44)	46.4 (7.1)
Central (800 ppm)	north	68.7 (14.4)	45.8 (3.0)	3.67 (0.35)	45.7 (3.7)
Central (800 ppm)	south	373 (69.7)	61.5 (14.1)	3.65 (0.00)	30.3 (0.6)
West (1200 ppm)	north	284 (69.5)	45.5 (11.2)	4.24 (0.46)	51.3 (2.9)
West (1200 ppm)	south	251 (17.6)	27.7 (3.5)	3.24 (0.29)	47.7 (3.8)
CO_2 effect		ns	$P = 0.10$	ns	$P = 0.028$
N–S effect		$P = 0.003$	ns	$P = 0.07$	$P = 0.058$
Interaction effect		$P = 0.051$	ns	ns	ns

Values are means and standard errors. Probability (P) values for two-way ANOVA are shown (ns, $P > 0.10$).

using the FastDNAml algorithm of BioEdit, derived from DNAML from the Phylip package (http://evolution.genetics.washington.edu/phylip.html); [9], using empirical base frequencies and a transition/transversion ratio of 2.0. Neighbor-joining analysis was performed with Phylip, using 100 bootstrap replicates, and the Jin-Nei method with a gamma factor of 0.1 to allow for different substitution rates between sites. The distance matrix generated with these parameters was also used to dereplicate the data set. Sequences not differing by more than 2% were considered to be identical operational taxonomic units (OTU). These data were used for rarefaction, richness, and diversity analysis using EstimateS software [5]. Differences among the 16S rRNA clone libraries were analyzed in two ways. The phylogenetic tree permutation (PTP) function of PAUP was used to compare the overall phylogenetic relationships between the communities [26]. PTP analysis does not rely on the frequencies of sequences in each library, and so is less subject to PCR bias [26, 27]. The hypothesis that phylogeny covaries with community type was tested by generating 10,000 randomly permuted trees and calculating the tree length needed to evolve the different communities. The tree length of the original data set was compared to this frequency distribution to produce a probability (P) value. The second approach was to compare differences in the frequency of major bacterial taxa within the clone libraries using analysis of variance (ANOVA). Where appropriate, values were log- or square-root-transformed before analysis.

Statistical Analysis. Measures of microbial activity were analyzed in a two-way ANOVA, with CO_2 treatment and the north–south gradient as the two factors. For the specific respiration data, a one-way ANOVA was used because some samples were lost. A two-way ANOVA would have been unbalanced and lacking in replicates for some cells. Data were log-transformed when appropriate. To test for a linear effect of CO_2, analysis of covariance (ANCOVA) was also used with the north–south gradient as a discrete factor, and atmospheric CO_2 concentration as a continuous variable. Factor analysis was performed on the 16S rRNA clone library data to describe covariations in the frequency of the three most abundant bacterial taxa (Acidobacteria, α- and β- Proteobacteria). The resulting factor [microbial activity factor (MCF)] was regressed on the microbial activity variables to relate community composition to function. Because process measurements and clone libraries were made on separate soil samples, for these regression analyses, the activity variables were averaged over the same sampling area used to construct clone libraries (i.e., mean values for each of the six plots were used). Similarly, factor analysis was used to produce a single factor [activity factor (AF)] from the plot means of several soil process rates. Glucose and Glutamate SIR were regressed on OM and water content to relate microbial activity to soil physical factors. For *a priori* tests (effects of CO_2, north vs south), probability (P) values are referred to as significant if $P < 0.05$, marginally significant if $0.05 < P < 0.1$, and not significant if $P > 0.1$. For *post-hoc* tests, Bonferonni correction was applied by dividing the critical alpha value ($\alpha = 0.05$) by the total number of tests. Statview (SAS Institute, Cary, NC, USA) and SYSTAT (SPSS Inc.) were used for statistical analyses.

Results

Environmental and Soil Characteristics. Based on data summed over the month of September, PAR was higher in the south plots than in the north (Table 1). There was also a decrease in PAR from east to west. Across both sampling dates, soil water content did not vary significantly between CO_2 treatments ($P = 0.317$) or along

Table 5. Pearson correlation coefficients among the frequencies of α, β, γ-Proteobacteria and Acidobacteria in the six clone libraries (probability values are shown in footnotes)

	α-Proteobacteria	*β-Proteobacteria*	*γ-Proteobacteria*
Acidobacteria	0.899[a]	−0.888[a]	−0.710[c]
α-Proteobacteria		−0.816[a]	−0.855[a]
β-Proteobacteria			0.804[b]

[a] $P < 0.05$.
[b] $P = 0.054$.
[c] $P = 0.114$.

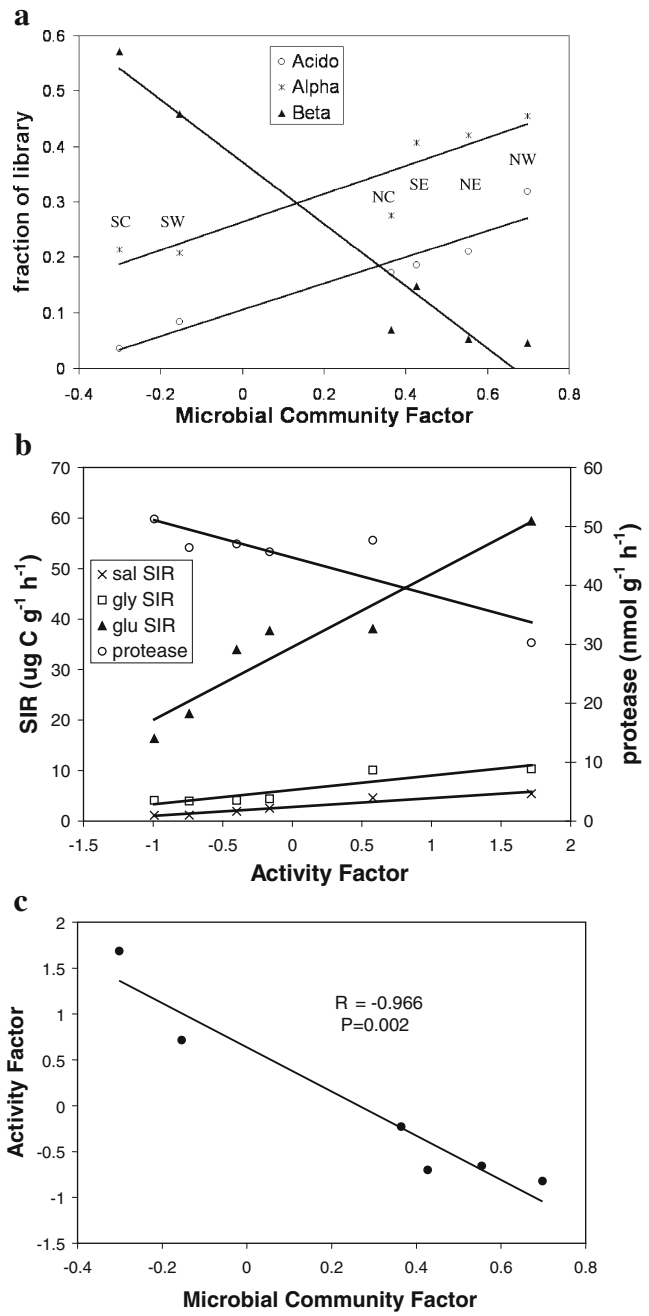

Figure 5. Results of factor analysis on bacterial clone libraries and soil process rates. (a) Relationships between the three most abundant bacterial lineages in the clone libraries and the "microbial community factor," which accounted for 91.2% of the variance in these variables. The six plots are labeled for reference (E: east, 400 ppm; C: central, 800 ppm; W: west, 1200 ppm; N: north; S: south). (b) Relationships between the soil process measurements (means for each plot) and the "activity factor," which accounted for 79.0% of the variance in these variables. (c) Correlation between the microbial community factor (described in a) and the activity factor (described in b).

the north–south gradient ($P = 0.127$). Soil OM did not vary significantly along the north–south gradient ($P = 0.132$), but was slightly higher in the west bay than in the east and central bays (overall CO_2 effect, $P = 0.032$; Table 1). Soil texture and pH did not vary greatly among the treatments (Table 1).

Bacterial Diversity. For the entire cottonwood plantation at B2L, the Chao1 estimate of OTU richness (at the 98% similarity level) was 411 ± 144 (Table 2). Chao1 estimates for individual plots show that the north plots appear to be more diverse than the south plots. The Chao1 estimates were highly uncertain, and in two libraries (NE, NW), no duplicate sequences were found and so no error estimates could be made. Therefore, the relative diversity of the plots is expressed as the number of unique (<98% similarity) OTU observed divided by the total number of sequences in each sample (Table 2). A small number of bacterial sequences were highly abundant, and appeared in two to five of the six clone libraries (Table 3). Of these six most abundant ribotypes, three were found only in the south plots of the elevated CO_2 bays (SC, SW). As discussed later, these two plots differed from the other four in microbial community composition and activity. There was no clear phylogenetic clustering of sequences by CO_2 treatment (Fig. 1). The PTP analysis verified this observation; whereas communities from different CO_2 treatments are nearly identical ($P = 0.953$), north communities were significantly different from south communities ($P = 0.013$). This north–south difference was entirely attributable to the south plots of the central and west (800 and 1200 ppm CO_2) bays ($P = 0.0001$). Fig. 1 shows four clusters that consisted exclusively of sequences from these two plots (dotted lines in the β-, γ-, and δ-Proteobacteria and in the Actinobacteria). Conversely, there were four clusters consisting solely of sequences from the other four plots (thick gray lines in the Acidobacteria, Actinobacteria, and in the α-Proteobacteria). Further PTP analysis confirmed that north and south plots within the east bay were quite similar to each other ($P = 0.929$) and to the north plots in the central and west bays ($P = 0.944$). These patterns are also apparent in the frequency of major bacterial taxa (Fig. 2). There were no consistent differences among CO_2 treatments (Fig. 2B), but there were significantly more β-Proteobacteria in southern plots and more Acidobacteria in northern plots (Fig. 2C). Again, it was the southern plots of the elevated CO_2 treatments that caused this effect (Fig. 2A); these two plots were rich in β-Proteobacteria, and depleted in Acidobacteria and α-Proteobacteria.

Microbial Biomass and Activity. Glucose SIR was essentially the only variable that showed a consistent and significant direct effect of elevated CO_2. Glucose SIR was

Table 6. Pearson correlation coefficients among the soil process variables comprised by the activity factor produced in factor analysis

	gly SIR	*glu SIR*	*Protease*
sal SIR	0.962[a]	0.896[a]	−0.729[b]
gly SIR		0.756[b]	−0.628[c]
glu SIR			−0.879[a]

[a] $P < 0.05$.
[b] $P \leq 0.01$.
[c] $P > 0.10$.

significantly increased by elevated CO_2 and in north plots in the two-way ANOVA (Fig. 3A). Fisher's protected least significant differences show that only the 1200-ppm treatment was significantly different from the others. ANCOVA revealed a significant linear correlation between glucose SIR and CO_2 concentration (P = 0.026), and the north–south factor was not significant in this analysis (P = 0.54). Salicylate, glycine, and glutamate SIR responses to elevated CO_2 were marginal (Fig. 3B–D). Salicylate and glycine SIR were significantly higher in south plots. As observed for bacterial diversity, large north–south differences in SIR were only found in the high CO_2 treatments (Fig. 3). This trend is reflected by ANCOVA for salicylate SIR, which showed a marginally significant $CO_2 \times$ N–S interaction (P = 0.057). Despite similar trends, ANCOVA for glycine and glutamate SIR were not significant (P = 0.131 and P = 0.159, respectively) Fungal hyphal length showed the same patterns as the salicylate, glycine, and glutamate SIR; there was no significant CO_2 treatment effect, but a marginally significant increase in the south plots (Fig. 4). *Post-hoc* ANOVA tests, using the Bonferonni correction, showed that the south high CO_2 plots (SC, SW) were significantly higher in salicylate SIR (P = 0.001), glycine SIR (P = 0.0014), and fungal hyphal length (P = 0.0043). Glutamate SIR was not significantly higher in this test (P = 0.057). Extracellular enzyme activities varied between treatments, but responded only marginally and/or nonlinearly to elevated CO_2 (Table 4). In the ANOVA, the CO_2 effect was significant for protease, although this was largely driven by low rates in the south 800-ppm plot.

Relationships Between Microbial Community Structure and Function. The four most abundant bacterial taxa, which comprised 78% of all sequences, were generally correlated with each other (Table 5). The con-

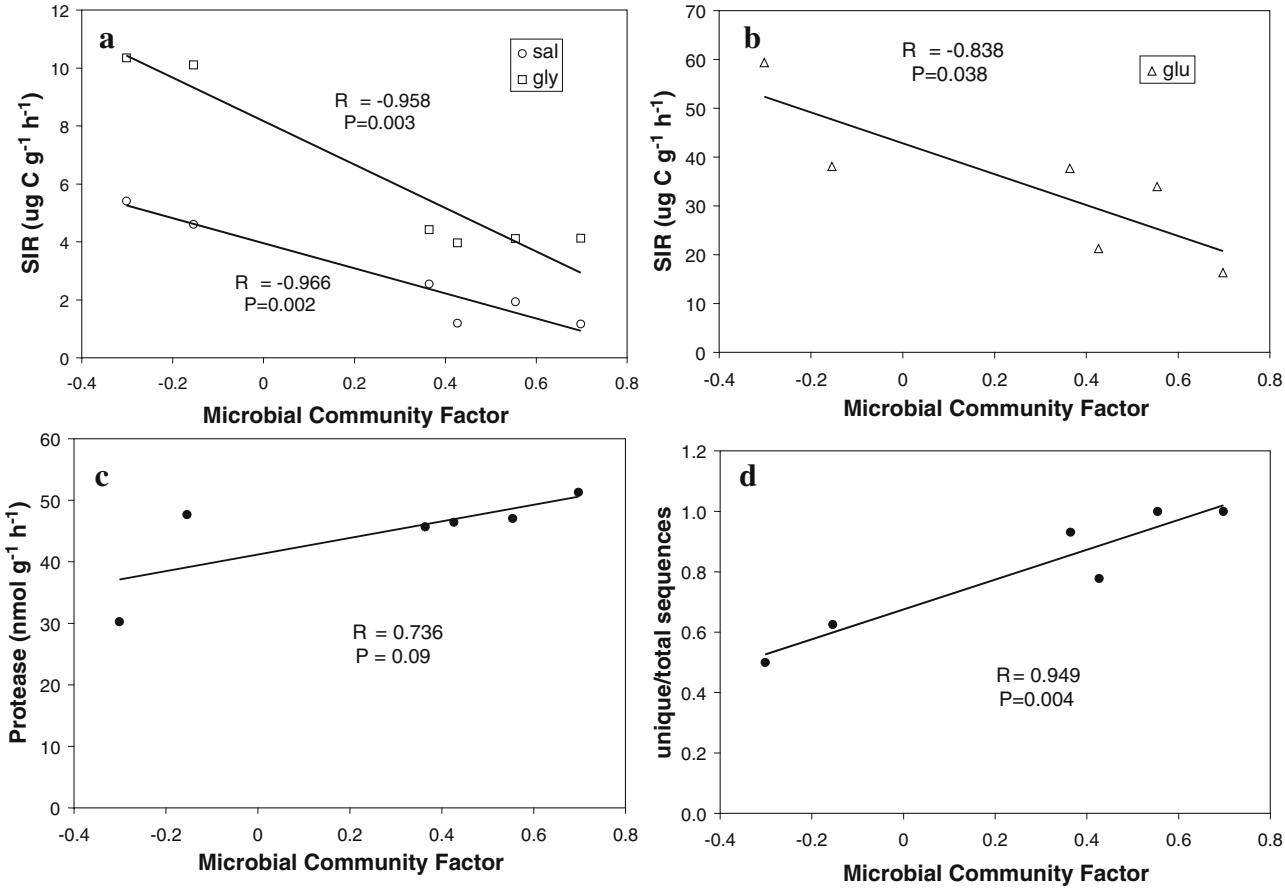

Figure 6. Correlations between the "microbial community factor" produced in factor analysis and the means of various soil processes: (a) glycine and salicylic acid SIR, (b) glutamate SIR, (c) protease rates, and with (d) diversity of the clone libraries, shown as the ratio of unique/total sequences.

sistent relationships between taxa allowed us, through factor analysis, to express the general composition of the bacterial community with a single continuous variable. The three most abundant groups (α-Proteobacteria, β-Proteobacteria, and Acidobacteria, representing 72% of all sequences) produced a single microbial community factor (MCF) that accounted for 91.2% of the variance in the data (Fig. 5a). As shown in Fig. 5a, soils with positive values of MCF are rich in β-Proteobacteria, whereas those with negative values are dominated by α-Proteobacteria and Acidobacteria. As previously noted, the south plots of the central and west bays (SC, SW) cluster apart from the others (Fig. 5a). Likewise, because several of the soil process variables were intercorrelated (Table 6), factor analysis allowed these variables to be distilled into a single activity factor (AF) (Fig. 5b). Increasing values of AF correspond to increased glu, gly, and sal SIR, and decreased protease rates. The MCF and AF variables, representing distilled descriptions of bacterial community structure and soil processes, respectively, are significantly correlated (Fig. 5c). For clarity, the individual soil processes that compose AF are shown in relation to the MCF (Fig. 6a–c). MCF was positively correlated with salicylate, glycine, and glutamate SIR. The negative relationship between MCF and protease was marginally significant. The relationship between MCF and the relative diversity (unique/total sequences) was highly significant (Fig. 6d), i.e., clone libraries dominated by β-Proteobacteria had fewer rare OTU and more abundant OTU, whereas the reverse was true in Acidobacterium-dominated samples. The ratio of unique/total sequences was also significantly negatively related to AF ($R = -0.861$, $P = 0.027$, data not shown). MCF was not significantly related to glucose SIR, soil water, or OM content, nor to activities of amylase, cellulase, or lignase. To help explain the lack of correlation between SIR using the two general heterotrophic substrates, glucose and glutamate, we regressed these variables on soil water

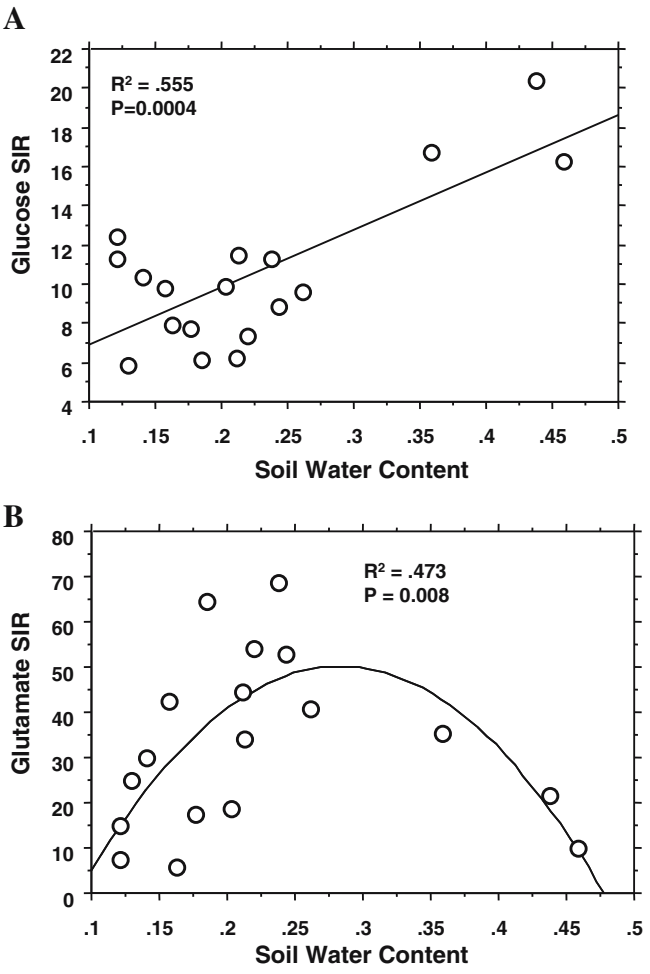

Figure 7. Relationships between field-collected soil water content and SIR for (A) glucose and (B) glutamate. The R^2 and P value are for the overall regression.

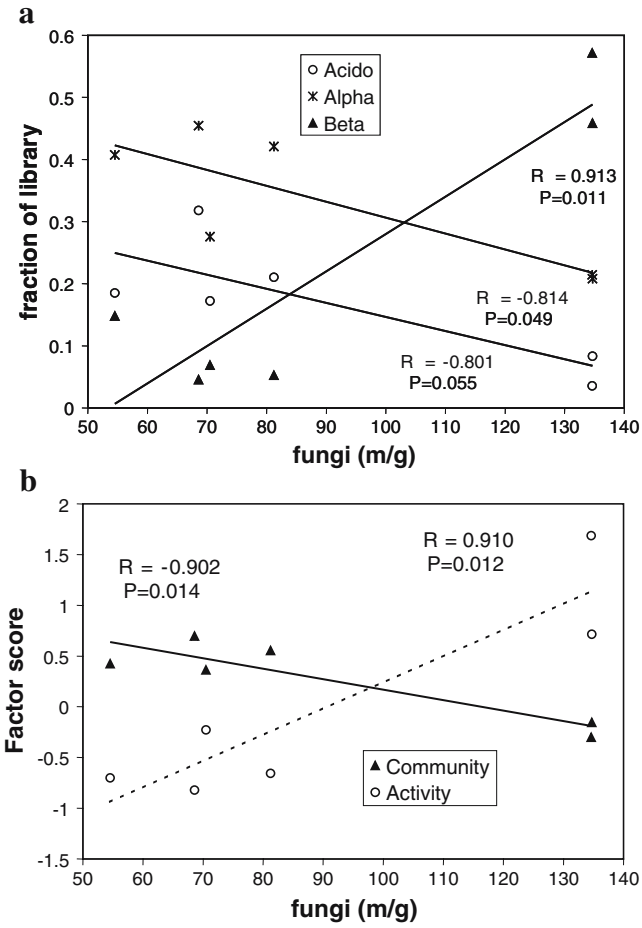

Figure 8. (a) Relationships between average fungal hyphal length and the abundance of the major bacterial taxa. (b) Relationships between average fungal hyphal length and the microbial community and activity factors produced by factor analysis (see Figures 2 and 5).

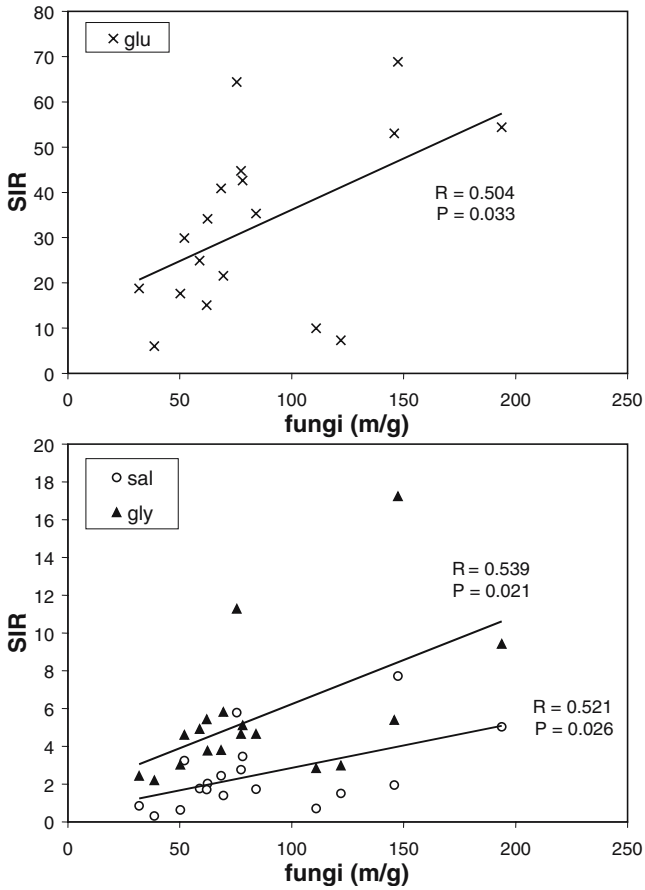

Figure 9. Regressions of SIR on fungal hyphal length.

content. Glucose SIR was linearly related to soil water content, whereas glutamate SIR fits a quadratic relationship (Fig. 7).

Fungal biomass also fits in well with the one-dimensional metrics of microbial community structure and activity: fungal hyphal length was generally correlated with the abundance of major bacterial taxa (Fig. 8a) and with both MCF and AF (Fig. 8b). Because measurements of fungal hyphae were made on individual soil samples, and not just soils pooled for molecular analysis, a more powerful regression analysis of the relationships between fungal biomass and soil process rates was possible. Fungal hyphal length was correlated to glu, gly, and sal SIR (Fig. 9). No other variables were significant in this analysis.

Discussion

Although B2L is an artificial ecosystem, the soil bacterial community was similar in composition to agricultural and natural soils [16]; the majority of sequences were α-Proteobacteria, β-Proteobacteria, and Acidobacteria, with the Gram-positive divisions, Acidobacteria and Firmicutes, also appearing. The Chao1 estimate of 411 unique 16S ribotypes (at the 98% similarity level) is lower than estimates for natural soils (e.g., 1166 for alpine soil [25]), but similar to estimates from other agricultural soils (467–590 [17]). The lower Chao1 estimates for the south plots (Table 2) and the relationship between microbial community structure and relative diversity (Fig. 6d) suggest that the Proteobacterial species that flourished in the south plots may have displaced some of the rarer species.

In terms of microbial activity, the most consistent effect of elevated CO_2 was an increase in glucose SIR. Glucose SIR is commonly used as an index of total heterotrophic microbial biomass and activity [2, 24, 42]. Increased heterotrophic microbial biomass is consistent with increased root biomass and soil respiration rates observed in the elevated CO_2 treatments of B2L [4]. In contrast to glucose SIR, the other effects of CO_2 were generally of marginal significance, and were driven by changes in the south sides of the elevated CO_2 treatment bays. Bacterial diversity, fungal biomass, and microbial activity were fairly uniform throughout the entire B2L cottonwood plantation, except for the south sides of the 800- and 1200-ppm treatments. This could indicate an interaction between elevated CO_2 and light. The only clear environmental difference between the north and south plots is solar exposure. Soil OM and water content were not significantly different along the north–south gradient, and pH and texture were uniform throughout the study area. Effects of elevated CO_2 on soil microbes are thought to be mediated through increased photosynthesis and rhizodeposition [21, 22, 31]. Therefore, it is understandable that the largest CO_2 effects occurred in the south ends of the bays, where light conditions were high enough for plants to capitalize on elevated CO_2 [33]. However, the north–south light gradient was subtle (7–22% increase in the 800- and 1200-ppm treatments, respectively), and our study was not designed to rigorously test the interactive effects of CO_2 and light. Therefore, it is possible that the north–south gradient resulted from some unknown, preexisting condition that was not detectable in our measurements of soil physical properties (OM, pH, texture, and water content). Hence, our hypotheses regarding elevated CO_2 were only partly confirmed: glucose SIR increased, as predicted, but patterns in the other measures of biomass, activity, and community structure could not be solely attributed to the CO_2 treatment.

Regardless of the original source of variability in soil properties within B2L, variations in soil process rates can be understood clearly in relation to variations in microbial community structure. The many recent studies of uncultured environmental microbial diversity have exposed large gaps in our knowledge of how microbial communities, and in fact, how most microbial species function [16, 36, 46]. Although many studies combine descriptions of uncultured diversity with measurements of soil processes, it is rare to show a numerical correlation between the abundance of bacterial taxa and soil process

rates. Because of the redundancy among the soil process variables and the consistent structure of the bacterial community, significant relationships between structure and function emerged, despite the low number of clone libraries. The present study indicates that Acidobacterial and α-Proteobacterial members of the B2L cottonwood plantation soil community are less physiologically active in terms of substrate use than β-Proteobacteria in this community. However, protease activity was higher in soils dominated by Acidobacteria and α-Proteobacteria. It is not surprising that β-Proteobacteria should be expert heterotrophs, given the physiological versatility found in this group (e.g., *Burkholderia*). Nor is it surprising that fungal biomass was also positively correlated with various heterotrophic activities. However, the positive correlation between Acidobacteria and α-Proteobacteria is contrary to the emerging generalization that these groups represent opposite ends of the metabolic spectrum; cultured Acidobacteria isolates generally grow slowly [41], and it has been suggested that Acidobacteria dominate low-nutrient soils, and α-Proteobacteria high-nutrient soils [46].

Studies of the effects of elevated CO_2 on soil microbial communities and activities have produced many conflicting results, with few common themes. Variation in microbial community composition is probably an important factor in these diverse results. Glucose SIR, a measure of heterotrophic microbial biomass and activity, responded consistently to CO_2 despite large variations in the microbial community. Such effects are commonly reported [18, 30, 31, 37, 50, 52]. In contrast, SIR of other substrates had more complex, nonlinear responses to CO_2 that depended on microbial community composition. These community-specific responses would more likely vary across ecosystems. The general heterotrophic substrates, glucose and glutamate, appeared to be degraded by different (although probably overlapping) sets of organisms. This is shown by the lack of correlation between these two processes, and that they responded differently to high soil water contents (Fig. 7). As oxygen diffusion is slowed down in wet soils, these differing relationships could indicate that glutamate is used predominantly by obligate aerobes, whereas glucose is used over a wider range of oxygen tensions. It is also possible that glucose and glutamate SIR differed because glucose SIR was N-limited. Whatever the source of these patterns, glutamate is clearly the preferred substrate in plots dominated by β-Proteobacteria and fungi.

Unlike most natural ecosystems, the B2L cottonwood plantation was generally free of environmental stresses. Such constraints on plant and microbial growth probably explain much of the variation between elevated CO_2 studies [10, 15, 20]. In the current study, there was a nonsignificant trend toward higher soil water contents in high CO_2 and north plots (Table 1). Given the strong linear dependence of glucose SIR on soil water content, it is possible that part of the effect of elevated CO_2 on glucose SIR was mediated by increased soil water content.

Like many other studies on the effects of elevated atmospheric CO_2 on soil microbes and processes, some responses to elevated CO_2 were intuitive and predictable, such as the increase in glucose SIR, whereas others, such as the other SIR, were more complex. However, these latter results could be accounted for by variations in the three most abundant bacterial taxa and by fungal biomass. It appears that a basic understanding of the soil microbial community is necessary for predicting how it will react to global change.

Acknowledgments

We gratefully acknowledge Spring Strahm, Laura Dane, and Richard Wilson for logistical and laboratory assistance, and Dr. Barry Osmond for his skilled coordination of research efforts at B2L. The comments of two anonymous reviewers were very helpful in revising this manuscript. Financial support was provided by the Packard Foundation and the Office of the Executive Vice Provost, Columbia University (Dr. Michael Crow). We especially would like to thank Mr. Edward P. Bass for making this study possible.

References

1. Amann, RI, Ludwig, W, Schleifer, K-H (1995) Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. Microbiol Rev 59: 143–169
2. Anderson, JPE, Domsch, KH (1978) A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biol Biochem 10: 215–221
3. Ausebel, FM (Ed.) (1994) Current Protocols in Molecular Biology. Wiley, New York
4. Barron-Gafford, G, Martens, D, Grieve, K, Biel, K, Kudeyarov, V, McLain, JE, Lipson, D, Murthy, R (2005) Growth of eastern cottonwoods (*Populus deltoides*) in elevated CO_2 stimulates stand-level respiration and rhizodeposition of carbohydrates, accelerates soil nutrient depletion, yet stimulates above and belowground biomass production. Global Change Biol 11: 1220–1233
5. Colwell, RK (1997) EstimateS: Statistical estimation of species richness and shared species from samples. Version 5. User's Guide and application published at: http://viceroy.eeb.uconn.edu/estimates
6. Diaz, S, Grime, JP, Harris, J, McPherson, E (1993) Evidence of a feedback mechanism limiting plant response to elevated carbon dioxide. Nature 364: 616–617
7. Ebersberger, D, Werrnbter, N, Niklaus, PA, Kandeler, E (2004) Effects of long term CO_2 enrichment on microbial community structure in calcareous grassland. Plant Soil 264: 313–323
8. Engel, VC, Griffin, KL, Murthy, R, Patterson, L, Klimas, CA, Potosnak, MJ (2004) Growth CO_2 modifies the transpiration response of *Populus deltoides* to drought and vapor pressure deficit. Tree Physiol 24: 1137–1145
9. Felsenstein, J (1989) PHYLIP-Phylogeny Inference Package (Version 3.2). Cladistics 5: 164–166
10. Field, CB, Lund, CP, Chiariello, NR, Mortimer, BE (1997) CO_2 effects on the water budget of grassland microcosm communities. Global Change Biol 3: 197–206

11. Grayston, SJ, Campbell, CD, Lutze, JL, Gifford, RM (1998) Impact of elevated CO_2 on the metabolic diversity of microbial communities in N limited grass swards. Plant Soil 203: 289–300
12. Griffiths, BS, Ritz, K, Ebblewhite, N, Paterson, E, Killham, K (1998) Ryegrass rhizosphere microbial community structure under elevated carbon dioxide concentrations, with observations on wheat rhizosphere. Soil Biol Biochem 30: 315–321
13. Griffin, KL, Turnbull, M, Murthy, R, et al. (2002) Leaf respiration is differentially affected by leaf vs stand-level night-time warming. Global Change Biol 8: 479–485
14. Hall, TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41: 95–98
15. Horz, HP, Barbrook, A, Field, CB, Bohannan, BJM (2004) Ammonia-oxidizing bacteria respond to multifactorial global change. PNAS 101: 15136–15141
16. Hugenholtz, P, Goebel, BM, Pace, NR (1998) Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. J Bacteriol 180: 4765–4774
17. Hughes, JB, Hellmann, JJ, Ricketts, TH, Bohannan, BJ (2001) Counting the uncountable: statistical approaches to estimating microbial diversity. Appl Environ Microbiol 67: 4399–4406
18. Hungate, BA, Holland, EA, Jackson, RB, Chapin, FS III, Mooney, HA, Field, HA (1997) The fate of carbon in grasslands under carbon dioxide enrichment. Nature 388: 576–579
19. Insam, H, Baath, E, Berreck, M, et al. (1999) Responses of the soil microbiota to elevated CO_2 in an artificial tropical ecosystem. J Microbiol Methods 36: 45–54
20. Körner, C (2000) Biosphere responses to CO2 enrichment. Ecol App 10: 1590–1619
21. Körner, C, Arnone, J III (1992) Responses to elevated carbon dioxide in artificial tropical ecosystems. Science 257: 1672–1675
22. Körner, C, Diemer, M, Schäppi, B, Niklaus, P, Arnone, J III (1997) The responses of alpine grassland to four seasons of CO_2 enrichment: a synthesis. Acta Oecologica 18: 165–175
23. Lipson, DA, Schadt, CW, Schmidt, SK (2002) Changes in microbial community structure and function following snow melt in an alpine soil. Microb Ecol 43: 307–314
24. Lipson, DA, Schmidt, SK, Monson, RK (1999) Links between microbial population dynamics and N availability in an alpine ecosystem. Ecology 80: 1623–1631
25. Lipson, DA, Schmidt, SK (2004) Seasonal changes in an alpine soil bacterial community in the Colorado Rocky Mountains. Appl Environ Microb 70: 2867–2879
26. Martin, AP (2002) Phylogenetic approaches for describing and comparing the diversity of microbial communities. Appl Environ Microb 68: 3673–3682
27. Mathieu-Daude, F, Welsh, J, Vogt, T, McClelland, M (1996) DNA rehybridization during PCR: the Cot effect and its consequences. Nucleic Acids Res 24: 2080–2086
28. Mayr, C, Miller, M, Insam, H (1999) Elevated CO2 alters community level physiological profiles and enzyme activities in alpine grassland. J Microbiol Methods 36: 35–43
29. Miller, DN, Bryant, JE, Madsen, EL, Ghiorse, WC (1999) Evaluation and optimization of DNA extraction and purification procedures for soil and sediment samples. Appl Environ Microb 65: 4714–4715
30. Mitchell, EAD, Gilbert, D, Buttler, A, Amblard, C, Grosvernier, P, Gobalt, JM (2003) Structure of microbial communities in Sphagnum peatlands and effect of atmospheric carbon dioxide enrichment. Microb Ecol 46: 187–199
31. Montealegre, CM, van Kessel, C, Russele, MP, Sadowsky, MJ (2002) Changes in microbial activity and composition in a pasture ecosystem exposed to elevated atmospheric carbon dioxide. Plant Soil 243: 197–207
32. Murthy, R, Griffin, KL, Zarnoch, SJ, et al. (2003) Response of carbon dioxide efflux from a 550 m^3 soil bed to a range of soil temperatures. Forest Ecol Management 178: 311–327
33. Murthy, R, Barron-Gafford, G, Dougherty, PM, et al. (2005) Increased leaf area dominates carbon flux response to elevated CO2 in stands of *Populus deltoides* (Bartr.) with higher fidelity in scaling leaf-stand processes under soil moisture stress. Global Change Biol 11: 716–773
34. Osmond, B, Ananyevw, G, Berry, J, et al. (2004) Changing the way we think about global change research: scaling up in experimental ecosystem science. Global Change Biol 10: 393–407
35. O'Neill, EG (1994) Response of soil biota to elevated atmospheric carbon dioxide. Plant Soil 165: 55–65
36. Pace, NR (1997) A molecular view of microbial diversity and the biosphere. Science 276: 734–740
37. Phillips, RL, Zak, DR, Holmes, WE, White, DC (2002) Microbial community composition and function beneath temperate trees exposed to elevated atmospheric carbon dioxide and ozone. Oecologia 131: 236–244
38. Prior, SA, Torbert, HA, Runion, GB, et al. (1997) Free air carbon dioxide enrichment of wheat: soil carbon and nitrogen dynamics. J Environ Qual 26: 1161–1166
39. Randlett, DL, Zak, DR, Pregitzer, KS, Curtis, PS (1996) Elevated atmospheric carbon dioxide and leaf litter chemistry: influences on microbial respiration and net nitrogen mineralization. Soil Sci Soc Am J 60: 1571–1577
40. Rillig, MC, Scow, KM, Klironomos, JN, Allen, MF (1997) Microbial carbon substrate utilization in the rhizosphere of *Gutierrezia sarothrae* grown in elevated atmospheric carbon dioxide. Soil Biol Biochem 29: 1387–1394
41. Sait, M, Hugenholtz, P, Janssen, PH (2004) Cultivation of globally distributed soil bacteria from phylogenetic lineages previously only detected in cultivation-independent surveys. Environ Microbiol 4: 654–666
42. Schmidt, SK (1992) A substrate-induced growth-response (SIGR) method for estimating the biomass of microbial functional groups in soil and aquatic systems. FEMS Microbiol Ecol 101: 197–206
43. Schortemeyer, M, Hartwig, UA, Hendrey, GR, Sadowsky, MJ (1996) Microbial community changes in the rhizospheres of white clover and perennial ryegrass exposed to free air carbon dioxide enrichment (FACE). Soil Biol Biochem 28: 1717–1724
44. Thompson, JD, Higgins, DG, Gibson, TJ (1994) Clustal-W—improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22: 4673–4680
45. Torbert, HA, Johnson, HB (2001) Soil of the intensive agriculture biome of Biosphere2. J Soil Water Conserv 56: 4–11
46. Torsvik, V, Øvreås, L (2002) Microbial diversity and function in soil: from genes to ecosystems. Curr Opin Microbiol 5: 240– 245
47. Tsai, YL, Olson, B (1992) Rapid method for separation of bacterial DNA from humic substances in sediments for polymerase chain reaction. Appl Environ Microbiol 58: 2292–2295
48. Van Ginkel, JH, Gorissen, A, Van Veen, JA (1996) Long term decomposition of grass roots as affected by elevated atmospheric carbon dioxide. J Environ Qual 25: 1122–1128
49. Van Ginkel, JH, Gorissen, A, Polci, D (2000) Elevated atmospheric carbon dioxide concentration: effects of increased carbon input in a *Lolium perenne* soil on microorganisms and decomposition. Soil Biol Biochem 32: 49–456
50. Williams, MA, Rice, CW, Owensby, CE (2000) Carbon dynamics and microbial activity in tallgrass prairie exposed to elevated CO_2 for 8 years. Plant Soil 227: 127–137
51. Zabel, B, Hawes, P, Hewitt, S, Marino, BDV (1999) Construction and engineering of a created environment: overview of the Biosphere 2 closed system. Ecol Eng 13: 43–63

52. Zak, DR, Pregitzer, KS, Curtis, PS, Teeri, JA, Fogel, R, Randlett, DL (1993) Elevated atmospheric CO_2 and feedback between carbon and nitrogen cycles in forested ecosystems. Plant Soil 151: 105–117
53. Zak, DR, Pregitzer, KS, Curtis, PS, Holmes, WE (2000) Atmospheric CO_2 and the composition and function of soil microbial communities. Ecol Appl 10: 47–59
54. Zak, DR, Pregitzer, KS, King, JS, Holmes, WE (2000) Elevated atmospheric CO_2, fine roots and the response of soil microorganisms: a review and hypothesis. New Phytol 147: 201–222