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Assessment of 10 years of CO_2 fumigation on soil microbial communities and function in a sweetgum plantation

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ABSTRACT

Increased vegetative growth and soil carbon (C) storage under elevated carbon dioxide concentration ($[CO_2]$) has been demonstrated in a number of experiments. However, the ability of ecosystems, either above- or belowground, to maintain increased C storage relies on the response of soil processes, such as those that control nitrogen (N) mineralization, to climatic change. These soil processes are mediated by microbial communities whose activity and structure may also respond to increasing atmospheric $[CO_2]$. We took advantage of a long-term (ca 10 y) CO_2 enrichment experiment in a sweetgum plantation located in the southeastern United States to test the hypothesis that observed increases in root production in elevated relative to ambient CO_2 plots would alter microbial community structure, increase microbial activity, and increase soil nutrient cycling. We found that elevated $[CO_2]$ had no detectable effect on microbial community structure using 16S rRNA gene clone libraries, on microbial activity measured with extracellular enzyme activity, or on potential soil N mineralization and nitrification rates. These results support findings at other forested Free Air $[CO_2]$ Enrichment (FACE) sites.

1. Introduction

Soil microorganisms control aspects of nutrient cycling; some of which are pivotal in the response of ecosystems to climate change. Examples include soil organic matter (SOM) transformations that may result in carbon (C) storage in stable soil pools and mobilization of nutrients that are required for C assimilation into plant biomass. However, the link between soil microbial community structure and ecosystem function is not well understood. Vegetation assimilates increasing amounts of C as atmospheric $[CO_2]$ increases (e.g., Norby et al., 2005), which may result in a flux of labile C to soils. Both leaf and root litter may decrease in quality (increased C:N ratios) (e.g., Cotrufo et al., 1994) and increase in quantity under elevated $[CO_2]$ via increased C allocation to litter production (e.g., Norby et al., 2004). In fact, several CO_2 enrichment experiments have shown an increase in soil organic C (Jastrow et al., 2005; Niklaus and Falloon, 2006), and many more report an increase in soil C inputs (Lichter et al., 2005; Hoosbeek et al., 2006). Leaf litter enters the soil profile by decomposing on the forest floor or by animal bioturbation, whereas roots decompose throughout the soil profile. The response of soil microbial communities to increasing soil C inputs remains inconclusive; some studies show changes in microbial community composition under elevated $[CO_2]$ (Lagomarsino et al., 2007, 2008), while others show no significant difference (Zak et al., 2000b; Grüter et al., 2006). If excess C is sequestered in soil C pools, forests may act as a negative feedback to increased global C emissions (Houghton et al., 1999).

Free air CO_2 enrichment (FACE) sites present a unique opportunity to study the effects of elevated $[CO_2]$ on ecosystem level processes in a natural setting without the interference of a chamber (Hendrey et al., 1999). An increase in net primary production (NPP) and higher C:N ratios have been observed under elevated $[CO_2]$ at the FACE site in Oak Ridge, TN (Norby and Iversen, 2006), but the system response to elevated atmospheric $[CO_2]$ is dominated by fine root production and turnover (Norby et al., 2004). Fine root turnover may provide a labile pool of easily metabolized C to microbial communities (Zak et al., 2000a). In fact, an increase in soil organic C has been observed in the top 5 cm of soil at these sites (Jastrow et al., 2005).

Given the increase in soil organic C at this site (Jastrow et al., 2005) and that soil microorganisms are considered C-limited (Zak et al., 1993), we predicted microbial community composition would

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change in response to increased soil C availability with increases in atmospheric [CO_2]. Specifically, we predicted heterotrophic bacterial groups and groups associated with cellulose breakdown may increase in abundance under elevated [CO_2]. Using rRNA gene libraries with universal 16S bacterial primers, we assessed a snapshot of the entire bacterial community and compared the relative proportions of different microbial groups under each treatment.

It is not the presence of specific microorganisms affecting soil nutrient transformations but rather the collective activity of soil microbial community. We expected to see changes in the activity of the soil microbial community as C becomes more available whether or not the bacterial community shifts in composition. For example, Phillips et al. (2002) found a 29% increase in metabolism of ^{13}C-cellobiose in soils exposed to elevated [CO_2] relative to ambient treatment soils. We used enzyme assays to assess the activity of six ecologically relevant enzymes. We expected the activity of important C transforming enzymes in soil to increase under elevated [CO_2].

Finally, we predicted that an influx of labile C to the soil would alter the rates of ecosystem functions such as nitrogen (N) mineralization. Understanding the response of N mineralization is especially important as continued increases in vegetative production require plant available N (Luo et al., 2004). However, the impact of increased soil C on N mineralization rates on ecosystem processes is very difficult to predict, and results vary greatly by experiment (e.g., Zak et al., 2000a). Increasing soil C could cause increased plant available N as activity is stimulated in the soil or decrease plant available N as microbes immobilize N while consuming increasing amounts of C. We measured potential N mineralization rates using laboratory incubations and predicted that plant available N would decrease in soils exposed to elevated [CO_2] relative to control soils.

We examined bacterial community structure using culture-independent molecular techniques (16S rRNA gene cloning analysis), microbial community function using extracellular enzyme activity, and N cycling rates using laboratory incubations at the Oak Ridge National Laboratory FACE site. Specifically, we asked three questions: 1) Are there shifts in the soil bacterial community structure associated with increased [CO_2]? 2) Does soil microbial functional activity increase with increases in soil C inputs associated with increasing atmospheric [CO_2]? and 3) Do elevated concentrations of atmospheric CO_2 decrease plant available N?

2. Materials and methods

2.1. Site description and experimental design

The FACE experiment on the Oak Ridge National Experimental Research Park (35°54′N 84°20′W) was established on an 8 y.o. sweetgum (*Liquidambar styraciflua* L.) plantation in 1998. Soils at the site are moderately well-drained with a soil pH of approximately 4.3 and are classified as Aquic Haplidult. FACE rings were constructed on four of five 25 m diameter plots (Norby et al., 2001). Two rings receive air with increased concentrations of CO_2 (544 parts 10^{-6} in 2006), two receive ambient air, and the fifth ring serves as a third ambient treatment without FACE apparatus. The mean annual temperature recorded in 2006 is 13.7 °C and annual precipitation is 1184 mm in 2006 evenly distributed throughout the year (Riggs et al., 2007). For a more detailed site description refer to Norby et al. (2001).

Mineral soil samples (0–15 cm) were collected with a 5 cm diameter hammer core in October 2006. Three cores were collected in each ring and homogenized in the field. Sub-samples were taken in the field and immediately frozen in liquid N_2 for molecular analysis. Field soils were sieved (2 mm) prior to laboratory analysis, and where appropriate data are shown on an oven-dry basis. Soil properties of these samples: total soil C, total soil N, and soil pH are described in Table 1.

2.2. Bacterial community structure: 16S rRNA gene libraries

2.2.1. DNA extraction and cloning

Soil samples collected from Rings 1 and 2 (CO_2-enriched) and Rings 4 and 5 (control) were chosen for molecular microbial analysis. Nucleic acids were extracted from 0.25 g of soil from each sample using the Powersoil DNA Isolation kit (MoBio Industries). Successful extraction was confirmed with the ND-1000 spectrophotometer at 260 nm (Nanodrop Technologies).

PCRs were performed on soil DNA using the 16S rRNA gene universal bacterial primers, 8F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1492R (5′-GGT TAC CTT GTT ACG ACT T-3′) (Lane, 1991). Reaction mixture included 2.5 μl 10× PCR buffer, 1.6 μl 25 mM $MgCl_2$, 2 μl 10 mg ml^{-1} BSA, 1.6 μl 25 mM dNTPs, 1 μl 20 pmol μl^{-1} each primer, 1 μl of undiluted soil DNA solution, 0.3 μl Taq-polymerase and H_2O to a final volume of 25 μl (9.5 μl). The reaction was carried out under the following conditions: 94 °C for 5 min followed by 30 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 2 min and finally 72 °C for 7 min in GeneAmp PCR system 9700 thermocyclers (PE Applied Biosystems). Positive and negative controls were used in all PCR reactions.

Electrophoresis was performed in 1% agarose in Tris-acetate EDTA buffer to confirm successful amplification and PCR products were extracted from the agarose gel using Qiagen Gel purification kit (Qiagen). PCR product (4 μl) was cloned using the TOPO-TA PCR 2.1 cloning kit (Invitrogen Corporation). Salt solution was omitted from the reaction and ligation at room temperature was extended to 30 min. White *Escherichia coli* colonies were picked and 16S cloned rRNA PCR products were lifted using M13 primers (M13F: 5′-GTA AAA CGA CGG CCA G-3′, M13R: 5′-CAG GAA ACA GCT ATG AC-3′) in a second PCR reaction under standard conditions. Successful M13 PCR amplification was screened using 1.5% agarose in Tris-Acetate EDTA buffer electrophoresis and 5 μl of product. M13 amplifications were purified with Millipore multiscreen HTS PCR filter plates (Millipore Corporation, Bellerica, MA, USA).

2.2.2. Sequencing and analysis

BigDye Terminator v3.1 (Applied Biosystems, Foster City, CA) Cycle Sequencing reactions were performed using a TA primer (Qiu et al., 2001) and the following master mix: 1 μl 5× sequencing buffer, 1.7 μl H_2O, 1 μl BigDye, 0.3 μl 20 pmol μl^{-1} TA primer, and 1 μl DNA template. Products were ethanol precipitated and resuspended in 10 μl of Hi-Di Formamide (Applied Biosystems) and run on an ABI PRISM 3700 DNA analyzer (Applied Biosystems). Sequences were edited in Sequencher program version 4.7 (Gene Codes Corporation) where the remnants of the vector and the low quality ends of the sequences were removed.

2.2.3. Taxonomic assignment and microbial community statistics

Clone analyses were performed using the Ribosomal Database Project II website (http://rdp.cme.msu.edu/) (Maidak et al., 2001). Sequences with less than 85% similarity to sequences found in the

Table 1
Elevated and Ambient [CO_2] effects on mineral soil (0–15 cm) total % N (*N*), Total % C, C:N, and pH. Data are mean values (SE). There were no significant differences between treatments ($P \leq 0.05$).

	%N	%C	C/N	pH
Ambient CO_2	0.13 (0.00)	1.39 (0.48)	10.85 (0.48)	4.36 (0.06)
Elevated CO_2	0.15 (0.02)	1.63 (0.21)	10.75 (0.21)	4.31 (0.13)

RDP database were compared using the NCBI BLAST database (http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi) (Ye et al., 2006), and sequences with ambiguous BLAST matches were considered unclassified. Sequences were aligned by Clustalx version 1.8 (Thompson et al., 1997). Phylogenetic trees were generated using neighbor-joining method in the BioEdit sequence alignment editor package (Hall, 1999). Bootstrap analysis was performed with 100 replicates. Edited sequences were submitted to Genbank (FJ620909–FJ621261). We constructed a neighbor-joining phylogenetic tree to detect differences in clustering of Acidobacteria between treatments.

Distance matrices were created using BioEdit and the *P*-test (Martin, 2002) was calculated for analysis of sequence similarity between treatments using the Unifrac web application available at http://bmf2.colorado.edu/unifrac (Lozupone and Knight, 2005). We used PRIMER (Primer-E ltd., San Francisco, CA, USA) to conduct an analysis of similarity (ANOISM) to compare the bacterial communities between treatments.

2.3. Soil microbial activity

Microbial functional activity was determined by assaying enzymes involved in C, N, and P transformations: β-1,4-glucosidase, cellobiohydrolase, β-xylosidase, α-1,4-glucosidase, N-acetyl-glucosaminidase, and phosphatase. The first four enzymes are associated with the breakdown of energy sources such as carbohydrates (O'Connell, 1987; Eivazi and Tabatabai, 1988; Sinsabaugh, 1994; Eivazi and Bayan, 1996; Boerner et al., 2000). N-acetyl-glucosaminidase is involved in the mineralization of N from chitin (Olander and Vitousek, 2000) and phosphatase is involved in the release of inorganic phosphorus (Eivazi and Tabatabai, 1977; Tarafdar et al., 1989). Enzymes were assayed using the 4-methylumbelliferyl (MUB)-linked substrates: β-D-glucoside, α-D-glucoside, 7-β-D-xyloside, β-D-cellobioside, N-acetyl-β-D-glucosaminide, and phosphate disodium salt (Saiya-Cork et al., 2002). Plates were scanned at an excitation of 355 nm and an emission of 460 nm on a Fluoroscan Ascent 2.4 utilizing Ascent software version 2.4.2. Enzyme activities were calculated as μmol product kg dry soil^{-1} h^{-1}.

2.4. Soil properties and potential N mineralization

Total soil C and N concentrations were determined using a Costech 4010 elemental combustion system (Costech Analytical Technologies, Inc. Valencia, CA). Soil pH was determined using a 1:2 suspension of air-dry soil to 0.01 M $CaCl_2$ solution (McLean, 1982, Orion 720A pH meter, Allometrics, Inc.). Gravimetric water content (GWC) was determined by drying (105 °C) each sample for 2 d. All relevant data are shown on a dry mass basis.

Potential soil N mineralization and nitrification were measured using laboratory incubations (Robertson et al., 1999). Samples were incubated (25 °C in the dark) in mason jars at field capacity. Samples were removed after 0 d, 31 d, and 60 d. Samples were extracted with 2 M KCl, and analyzed on a Lachat QuikChem 8500 Series FIA+ Analyzer (Lachat Instruments, Hatch Co., Loveland, CA, USA).

2.5. Statistical analysis

We used one-way analysis of variance tests (ANOVA and MANOVA) to test for differences between ambient and elevated CO_2 soil properties (%C, %N, C:N, pH), enzyme activity, potential net nitrification, and potential net N mineralization. Where needed, samples were log transformed prior to analyses to improve normality and homogeneity of variances; non-transformed data are shown in all tables and figures. Statistics were conducted with JMP 5 statistical software with significance defined as $P \leq 0.05$ (SAS Institute).

3. Results

3.1. Bacterial community structure

A total of 83, 95, 82, and 83 clones were obtained for Rings 1, 2, 4, and 5, respectively from 16S rRNA gene clone libraries. Analysis of variance revealed no significant differences between treatments ($R = 0$, $P = 0.333$). Fifteen to twenty percent of clones from each ring were defined as unclassified. These clones represented poor matches to cultured organisms but often had high resemblance to previously sequenced environmental clones in the NCBI database. This signifies that many of the clones sequenced in this study are common to soils in different environments.

Acidobacteria and Proteobacteria represented the two most common phyla of bacteria. Proteobacteria accounted for 24% of bacterial clones from elevated treatments and 23% of clones from the ambient treatment (Fig. 1). Five classes of Proteobacteria phyla (α-, β-, δ-, γ-, and ε-proteobacteria) are established, and we determined the phylogenic affiliation of bacterial clones obtained in this study to these five classes (Fig. 2). The most common group of

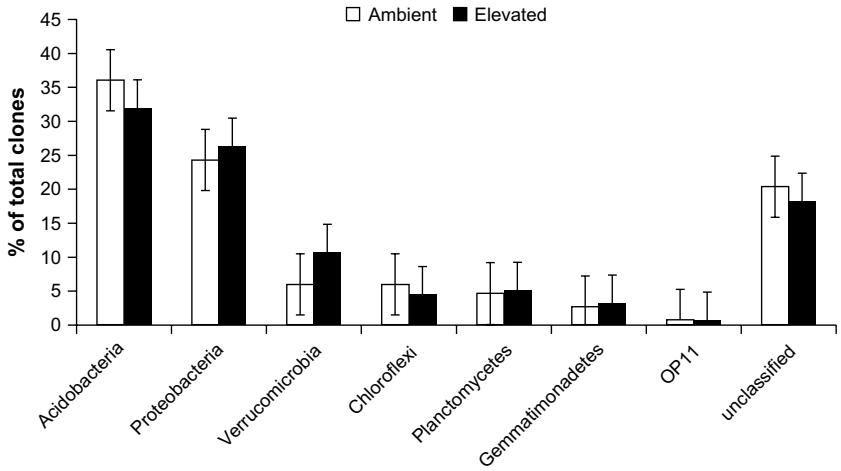

Fig. 1. Distribution of bacterial clones from 16S rRNA gene clone libraries is shown by phylum. Numbers are represented as a percentage of total clones in elevated [CO_2] (black) and ambient [CO_2] (white) rings ± standard error.

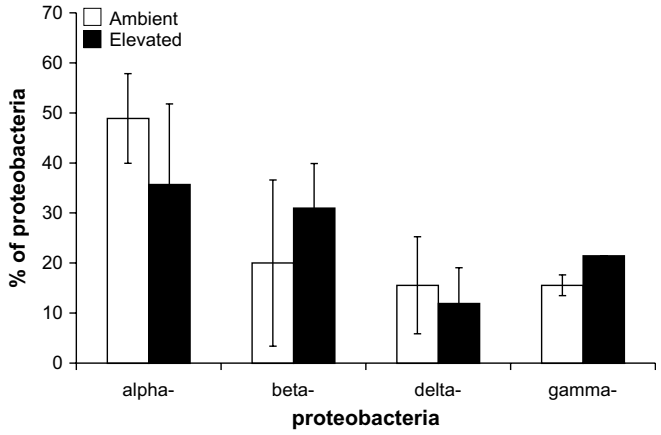

Fig. 2. Distribution of proteobacterial sequences in 16S rRNA clone libraries by class. Numbers are represented as a percentage of total clones from all classes of Proteobacteria observed in elevated [CO_2] (black) and ambient [CO_2] (white) rings ± standard error. Only clones classified with a certainty of 70% or above are represented.

Proteobacteria within these soils was α-proteobacteria (35–50% of Proteobacteria), and the *Rhizobiales*-like clones were common within this group. β-proteobacteria were the second most prominent group (20–30% of Proteobacteria), while δ-proteobacteria and γ-proteobacteria each represented about 15–20% of Proteobacteria within each treatment. No ε-proteobacteria were detected. No significant changes were observed in the grouping of Proteobacteria between treatments.

The other dominant phyla, Acidobacteria, represented 29% and 34% of clones from elevated and ambient [CO_2] treatments respectively. This group is more recently established (Barns et al., 2007), and the majority of sequences are from uncultivated representatives. However, no clustered pattern was observed for the Acidobacteria sequences, as clones from each ring were present in all branches of the tree (data not shown). Unifrac comparison of the sequences classified as Acidobacteria revealed no significant differences in Acidobacteria diversity between treatments ($P \geq 0.1$).

The third most common phylum, Verrucomicrobia, is commonly found in acidic soils and sediments. It represented approximately 10% of clones from elevated [CO_2] soils and 5% of clones from ambient [CO_2] soils. The remaining phyla represented in this study accounted for less than 5% of clones and included Actinobacteria, Bacteriodetes, Chloroflexi, Firmicutes, Gemmatimonadetes, OP11, and Plantomycetes, all commonly found in soils.

3.2. Microbial activity

None of the six extracellular enzyme assays reveal significant differences between elevated and ambient [CO_2] treatments (Table 2). The activities of extracellular enzymes measured in this study are within the range of rates reported in the literature (Larson et al., 2002; Sinsabaugh et al., 2003; Finzi et al., 2006; Billings and Ziegler, 2008). Of the enzymes involved in C transformations, β-1,4-glucosidase, cellobiohydrolase and β-xylosidase displayed lower activity in elevated [CO_2] treatments as compared to control treatments while lower activity rates were observed in control treatments as compared to elevated [CO_2] treatments for α-1,4-glucosidase (Table 2).

3.3. Potential soil N mineralization

Both elevated and ambient [CO_2] treatment soils showed an initial decrease in potential net mineralization after 30 days and an overall increase after 60 days (Fig. 3A). Mineralization rates were not significantly different in ambient relative to elevated treatments ($P = 0.104$). Nitrification rates also showed an overall increase after 60 days (Fig. 3B). Potential net nitrification did not differ between elevated and ambient treatments ($P = 0.119$).

Table 2
Mean (±1 standard error) enzyme activities in ambient and elevated CO_2 soils. There were no significant differences between treatments ($P \geq 0.05$). *$n = 1$ for N-acetyl-glucosaminidase under ambient CO_2.

Enzyme (μmol kg^{-1} h^{-1})	Ambient CO_2	Elevated CO_2
Ligno-celluloytic enzyme activities		
α-1,4-glucosidase	1.0 ± 0.9	2.1 ± 0.8
β-1,4-glucosidase	145.7 ± 18.6	129.5 ± 7.8
Cellobiohydrolase	21.6 ± 5.0	18.3 ± 4.1
β-xylosidase	26.0 ± 2.4	24.5 ± 8.6
Nutrient mineralizing enzyme activities		
N-acetyl-glucosaminidase	48.0*	63.8 ± 6.7
alkaline phosphatase	332.4 ± 56.3	234.8 ± 7.6

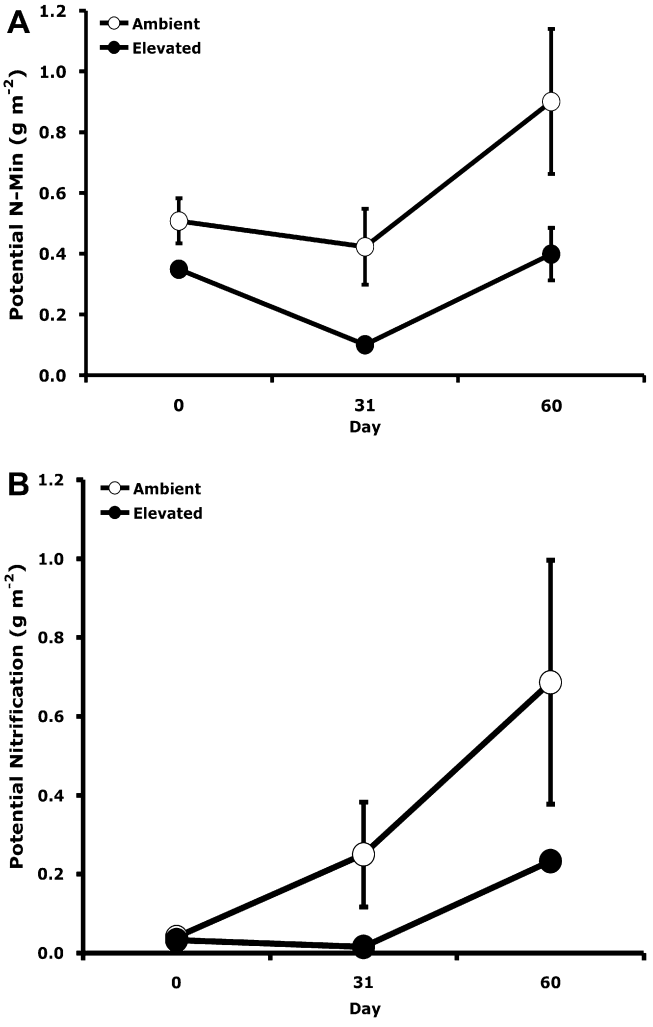

Fig. 3. Potential net N mineralization (A) and net nitrification (B) rates from elevated [CO_2] (black) and ambient [CO_2] (white) treatments calculated from laboratory incubations as A: total $NH_4 + NO_3$ (gm^{-2}) and B: total NO_3 (gm^{-2}) ± standard error. Potential net N-minerlaization rates were calculated as 0.0066 g^{-1} m^{-2} d^{-1}(ambient [CO_2]) and 0.0008 g^{-1} m^{-2} d^{-1}(elevated [CO_2]). Potential net nitrification rates were calculated as 0.0107 g^{-1} m^{-2} d^{-1} (ambient [CO_2]) and 0.0033.

4. Discussion

We predicted that increased C allocation to fine roots and the turnover of these roots observed by others in this system (Iversen et al., 2008) would change the soil environment and thus the microbial community and its activity. Specifically we expected to observe changes in soil bacterial community structure that could be associated with a shift away from oligotrophic organisms towards generalist heterotrophic organisms as C input increased. We also predicted the microbial community as a whole would show higher activity rates in response to higher C inputs and finally we predicted changes in microbial community structure and function would alter N transformation rates. The methods we used, 16S rRNA gene clone libraries, extracellular enzyme activity, and laboratory incubations, are commonly used as experimental tools to test hypotheses similar to ours (Ebersberger et al., 2003; Sinsabaugh et al., 2003; Lipson et al., 2006), and we did not detect any significant changes. The response of these variables to increased C inputs may be occurring slowly or at rates too low to detect using these relatively coarse methods. Changes in soil C content have been observed in the top 5 cm of soil (Jastrow et al., 2005), and any shifts in community structure within this surface layer of soil could have been diluted by our combining soil from 0 to 15 cm. Additionally, our chosen sampling methods would not have allowed us to access any changes that may have occurred with increased root production deeper in the soil profile.

4.1. Are there shifts in the soil bacterial community structure associated with increased [CO_2]?

In our soils the phylum Acidobacteria was the most abundant 32–36% of total sequences, followed by Proteobacteria and Verrucomicrobia. These results agree well with a recent synthesis of soil bacterial community composition studies by Janssen (2006) that showed the most abundant phylum commonly found in soil bacterial communities in general is Proteobacteria (mean 40%), followed by Acidobacteria (mean 20%), then Actinobacteria (mean12%) and Verrucomicrobia (mean 5%). The abundance of Acidobacteria we observed falls in the high end of Acidobacteria abundance found via 16S clone libraries in soils, but within the range of previously reported data (Janssen, 2006). Acidobacteria tend to prefer oligotrophic soils (Fierer et al., 2007), and they do not seem to be out competed in soils of high [CO_2] plots despite the increased flux of C. This may be related to heterogeneity within the soil or to the persistence of micro-niches of nutrient poor environments.

We did not detect changes in microbial community structure at the phylum level. Previous studies of bacterial diversity at FACE sites have reported a lack of significant change in microbial community composition (Zak et al., 2000b; Lipson et al., 2005). However, some studies have shown a shift in microbial community structure as a response to elevated [CO_2] in grasslands (Schortemeyer et al., 1996; Drissner et al., 2007) or in rhizosphere communities (Marilley et al., 1999). At a FACE site in a quaking aspen stand Lesaulnier et al. (2008) found no changes in bacterial community structure at the phylum level; however, they observed significant changes in the number of operational taxonomic units (OTUs) within some Proteobacterial groups and Actinobacterial groups. The methods we used were aimed at detecting changes in microbial community structure at the phylum level and are not refined enough to detect changes in the abundance of OTUs within phyla.

The uniformity of the bacterial community structure under elevated [CO_2] treatments despite changing climatic conditions and soil chemistry may be related to a low background diversity in the soils at this site (Waldrop and Firestone, 2006). Soils exposed to more diverse conditions over time may experience a more profound shift in microbial community structure. The microbial techniques we used were not absolutely quantitative but rather aimed at uncovering any relative abundance trends towards structural changes in the community. However, there may be quantitative changes in bacterial community that are not detected by these methods and may affect aspects of community and ecosystem function.

4.2. Does the functional activity of the soil bacterial community increase with increases in soil C inputs under elevated atmospheric [CO_2]?

We did not detect any changes in enzyme activity in soils from enriched [CO_2] plots when compared to ambient plots. While some FACE studies have shown significantly greater activity of some enzymes (Larson et al., 2002; Finzi et al., 2006), other studies have observed no difference in extracellular enzyme activity. For example no changes were found at this site five years prior to this study (Sinsabaugh et al., 2003) as well as at other FACE sites (e.g., Phillips et al., 2002). The activity of extracellular enzymes measured in this study are within the range of those reported previously at Oak Ridge FACE (Sinsabaugh et al., 2003) as well as at other FACE sites (Larson et al., 2002; Finzi et al., 2006).

Studies measuring annual variation in enzyme activity show seasonal fluctuation in rates (Ebersberger et al., 2003; Larson, 2002). In studies that have observed significant increased enzyme activity under elevated [CO_2], the samples were collected in the spring and summer seasons (Larson et al., 2002; Ebersberger et al., 2003). Since our samples were taken during the fall as plants began senescence, we may not be observing the highest rates of enzyme activity at this site.

Finzi and Schlesinger (2003) found enzyme activities in the O-horizon to be about an order of magnitude higher than activities in the top 15 cm of mineral horizon. Changing C inputs to soils at our site may be coming from changes in leaf quantity or fine root quantity at depth (Iversen et al., 2008). We expected to see changing enzyme activity throughout the soil profile as larger compounds in the leaves broke down and organic material moved down throughout the soil profile. We did not detect changes in enzyme activity in soils pooled for 0–15 cm. It appears that changes in aboveground inputs have not yet altered enzyme activity in the top 15 cm of soil. It is possible that changes may be occurring on a finer scale such as within the top 5 cm or deep within the soil profile. Our sampling from 0 to 15 cm could mask those fine-scale differences.

4.3. Do elevated concentrations of atmospheric CO_2 decrease plant available N?

We expected to see decreased plant available N under elevated [CO_2] as increased C inputs stimulated microbial activity and microbial immobilization occurred. Trends towards a decrease in potential net N mineralization rates under elevated [CO_2] can indicate either a decrease in gross N mineralization rates, increased rates of microbial N-immobilization (Zak et al., 2000a) or a shift in the quality of SOM (Billings and Ziegler, 2005). No change in root [N] has been observed at this site (Norby and Iversen, 2006). We would expect the trend towards a decrease in rates of net N mineralization observed in elevated [CO_2] soils relative to ambient [CO_2] soils to be a result of increased immobilization; however, our enzyme activity results indicate that increased fine root inputs have not stimulated the measured functional microbial activities in [CO_2] enriched plots.

Previous studies at other FACE sites have also shown no change in N cycling rates (Zak et al., 2003; Finzi et al., 2006). There is no consensus on the direction and magnitude of change in N cycling, if it exists, under elevated atmospheric [CO_2]. Billings and Ziegler (2005) did observe a decline in potential N mineralization, but these results were cumulative after long-term laboratory incubations, and the results may not reflect field conditions. Zak et al. (2000b) suggest that in soils where the ratio of microbial C pool to fine root C inputs is very high, the increased C from fine root growth may not affect the soil microbial community and nutrient cycling. Studies detecting an effect of [CO_2] on N cycling rates may have high root proliferation or low soil C, thus increased root growth could contribute to a greater percentage of total soil C. This is one possible scenario that may explain the lack of change in N mineralization rates at this site.

5. Conclusion

The increased C inputs to the soil at this site are occurring via increased leaf litter, which first affects the O-horizon of the soil, and via increased fine root proliferation throughout the soil profile, especially at depth (Iversen et al., 2008). Contrary to our expectations, we found that increased soil C inputs are not affecting microbial community structure, enzyme activity, or N cycling within the top 15 cm of the soil profile. However, if biogeochemical cycling in soils responds in areas of high microbial concentration such as the rhizosphere or in the top fraction of the soil, or if root exudation and root turnover rates are increasing C allocation to deep soils, there may be important localized effects on nutrient cycling which would not have been observable in this study where bulk soil was combined.

Since no effects (adverse or otherwise) have been observed on bacterial communities and functional activity in this study, increased C inputs may continue to accumulate within the soil. While more extensive sampling may be able to detect small changes in soil community structure and function, it appears that these changes will not be dramatic enough to limit biomass production or deplete soil C pools at present, after about a decade of enriched [CO_2] fumigation.

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