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Elevated Carbon Dioxide Alters the Structure of Soil Microbial Communities

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Pyrosequencing analysis of 16S rRNA genes was used to examine impacts of elevated CO_2 (eCO_2) on soil microbial communities from 12 replicates each from ambient CO_2 (aCO_2) and eCO_2 settings. The results suggest that the soil microbial community composition and structure significantly altered under conditions of eCO_2, which was closely associated with soil and plant properties.

The concentration of atmospheric CO_2 is increasing, largely due to human activities, and it is projected to reach 700 μmol/mol by the end of this century (15, 17). Although it is well established that elevated CO_2 (eCO_2) stimulates plant growth and primary productivity (2, 19, 23), the impact of eCO_2 on soil microbial communities remains poorly understood (5, 7, 11–14, 18, 22, 28). Pyrosequencing of 16S rRNA genes has been used to analyze the diversity, composition, structure, and dynamics of microbial communities from different habitats, such as soil (1, 6, 10, 24, 25, 27), water (4, 21), fermented foods (16), and human gut (26). In this study, we aimed to (i) examine effects of eCO_2 on the taxonomical diversity, composition, and structure of soil microbial communities and (ii) link soil and plant properties with the microbial community composition and structure by the use of tag-encoded pyrosequencing of 16S rRNA genes.

To address those issues, this study was conducted within the BioCON (Biodiversity, CO_2, and Nitrogen) experiment site located at the Cedar Creek Ecosystem Science Reserve in Minnesota (23). For this study, 24 soil samples (12 from ambient CO_2 [aCO_2] settings, 12 from eCO_2 settings, and all with 16 plant species and without an added nitrogen supply) were collected in July 2007. eCO_2 plots have been treated since 1997 during the plant growing season (May to October) every year, and each sample was composited from five soil cores at a depth of 0 to 15 cm. Details are provided in Materials and Methods in the supplemental material.

The V4 and V5 regions of 16S rRNA genes were amplified using a conserved primer pair with a unique 6-mer oligonucleotide sequence (barcode) at the 5′ end for each sample (see Table S1 in the supplemental material). After preprocessing of all reads, 30,008 and 29,091 high-quality sequences were obtained for aCO_2 and eCO_2 samples, respectively. The numbers of sequences from different samples ranged from 1,854 to 3,087 for aCO_2 samples and from 1,698 to 3,299 for eCO_2 samples (see Table S2 in the supplemental material). All sequences were aligned using the RDP Infernal Aligner, and the complete linkage clustering method was used to define operational taxonomic units (OTUs) using 97% identity as a cutoff, resulting in 2,527 and 2,354 OTUs for aCO_2 and eCO_2, respectively (see Table S2 in the supplemental material). The average Shannon indices were 6.58 and 6.51 for aCO_2 and eCO_2 samples, respectively (see Table S2 in the supplemental material). However, no significant ($P > 0.05$) differences were seen in the numbers of sequences, OTUs, or Shannon diversity index between aCO_2 and eCO_2 samples. In total, 3,500 OTUs were detected for at least 2 of 12 aCO_2 or eCO_2 samples, phylogenetically deriving from one known archaeal phylum and 16 known bacterial phyla as well as unclassified phylotypes. Most phylotypes were detected at both aCO_2 and eCO_2, with only a few detected at only aCO_2 or eCO_2 (see Table S3 and Fig. S1 in the supplemental material). At the phylum level, 811 (23.2%) OTUs were derived from *Proteobacteria*, a phylum with the highest number of detectable OTUs, followed by *Actinobacteria* (596; 17.0%), *Bacteroidetes* (448; 12.8%), and *Acidobacteria* (369; 10.5%) (Table 1).

To examine whether eCO_2 affects the taxonomical composition and structure of soil microbial communities, principal coordinate analysis (PCoA) was performed with the relative abundance values of 454 pyrosequencing data. Two distinct clusters were formed, and aCO_2 samples were well separated from eCO_2 samples (Fig. 1). The results of three nonparametric multivariate statistical tests, ANOSIM (8), Adonis (3), and MRPP (20), showed significant differences ($P = 0.001$ and $\delta = 0.481$, $P = 0.003$ and $R = 0.209$, and $P = 0.001$ and $R^2 = 0.082$, respectively) based on the abundance of all OTUs detected at aCO_2 and eCO_2, and such significances ($P < 0.05$) were also observed at the phylum level, including *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, DP10, *Proteobacteria*, TM7, and WS3 (see Table S4 in the supplemental material). The results indicated that the overall taxonomic composition and structure of soil microbial communities shifted in response to eCO_2.

To further examine effects of eCO_2 on microbial communities, we analyzed both significantly changed and unique OTUs. Among

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TABLE 1 Numbers of OTUs detected under aCO_2 and eCO_2 conditions

Domain and phylum	No. of OTUs			
		Avg[b]		
	Total[a] (%)	aCO_2	eCO_2	*P* (unpaired *t* test)
Archaea				
Crenarchaeota	10 (0.28)	4.42 ± 1.73	3.17 ± 1.59	0.08
Unclassified	1 (0.03)			
Bacteria				
Acidobacteria	369 (10.50)	112.33 ± 20.08	91.08 ± 19.11	0.01
Actinobacteria	596 (17.00)	179.83 ± 26.18	179.25 ± 25.52	0.96
Bacteroidetes	448 (12.80)	105.58 ± 24.54	100.17 ± 21.07	0.57
BRC1	2 (0.57)	0.17 ± 0.39	0.25 ± 0.45	0.63
Chlamydiae	36 (1.03)	3.08 ± 2.39	3.92 ± 3.00	0.46
Chloroflexi	94 (2.69)	17.33 ± 4.23	14.92 ± 6.04	0.27
Cyanobacteria	3 (0.86)	0.75 ± 0.87	0.17 ± 0.39	0.04
Firmicutes	118 (3.4)	28.58 ± 5.65	24.33 ± 5.99	0.09
Gemmatimonadetes	86 (2.46)	22.83 ± 5.81	17.92 ± 5.52	0.05
Nitrospirae	6 (0.17)	3.00 ± 1.48	2.00 ± 1.04	0.07
OP10	8 (0.23)	1.42 ± 1.51	0	0.00
Planctomycetes	251 (7.17)	42.00 ± 8.82	39.67 ± 12.37	0.60
Proteobacteria	811 (23.2)	207.08 ± 30.79	220.33 ± 30.22	0.30
TM7	52 (1.49)	7.83 ± 3.79	6.25 ± 4.11	0.34
Verrucomicrobia	127 (3.63)	29.33 ± 8.32	22.92 ± 8.01	0.07
WS3	6 (0.17)	1.00 ± 0.74	0.83 ± 1.19	0.68
Unclassified	454 (13.00)			
Total unclassified	22 (0.63)			
Total	3,500 (100)	846.75 ± 108.71	800.5 ± 122.03	0.34

[a] Data represent total numbers of OTUs detected by pyrosequencing across all 24 samples.
[b] Data represent average numbers of OTUs detected using 12 samples under aCO_2 or eCO_2 conditions.

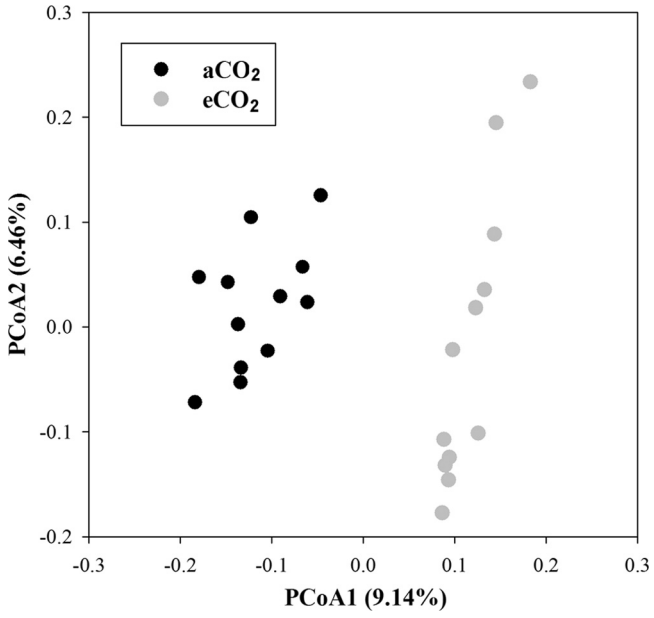

FIG 1 Principal coordination analysis (PCoA) of soil microbial community structure based on the relative abundances of OTUs detected by 454 pyrosequencing.

3,500 OTUs detected, 1,381 were shared by aCO_2 and eCO_2 samples, and 1,146 and 973 unique OTUs were detected at aCO_2 and eCO_2, respectively. For those shared OTUs, 27 were significantly ($P < 0.05$) decreased and 36 were significantly ($P < 0.05$) increased at eCO_2 (see Table S5 in the supplemental material). For example, 26 significantly changed OTUs were found in *Actinobacteria*, with 7 decreased and 19 increased at eCO_2 (see Table S5 and Fig. S2 in the supplemental material). Also, 11 OTUs were significantly ($P < 0.05$) changed at eCO_2, with 9 increased and 2 decreased in *Proteobacteria*, and 5, 3, 2, and 1 OTUs were derived from *Alpha-*, *Beta-*, *Delta-*, and *Gammaproteobacteria*, respectively (Fig. 2; see also Table S5 in the supplemental material). In addition, significantly ($P < 0.05$) changed OTUs in other phyla, such as *Acidobacteria*, *Bacteroidetes*, and *Planctomycetes*, were observed (Fig. 2; see also Table S5 in the supplemental material). A large percentage (60.5%) of unique OTUs was detected by pyrosequencing, with 1,146 (32.7%) at aCO_2 and 973 (27.8%) at eCO_2, and those OTUs were largely derived from the most abundant phyla (see Table S5 in the supplemental material). For example, 213 and 243 unique OTUs were detected at aCO_2 and eCO_2, respectively, for *Proteobacteria*, and 170 and 150 for *Actinobacteria*. The analysis of significantly changed and unique OTUs confirmed that the taxonomic composition and structure of soil microbial communities significantly changed at eCO_2.

To understand whether microbial populations differentially respond to eCO_2, detected OTUs were mapped to their associated

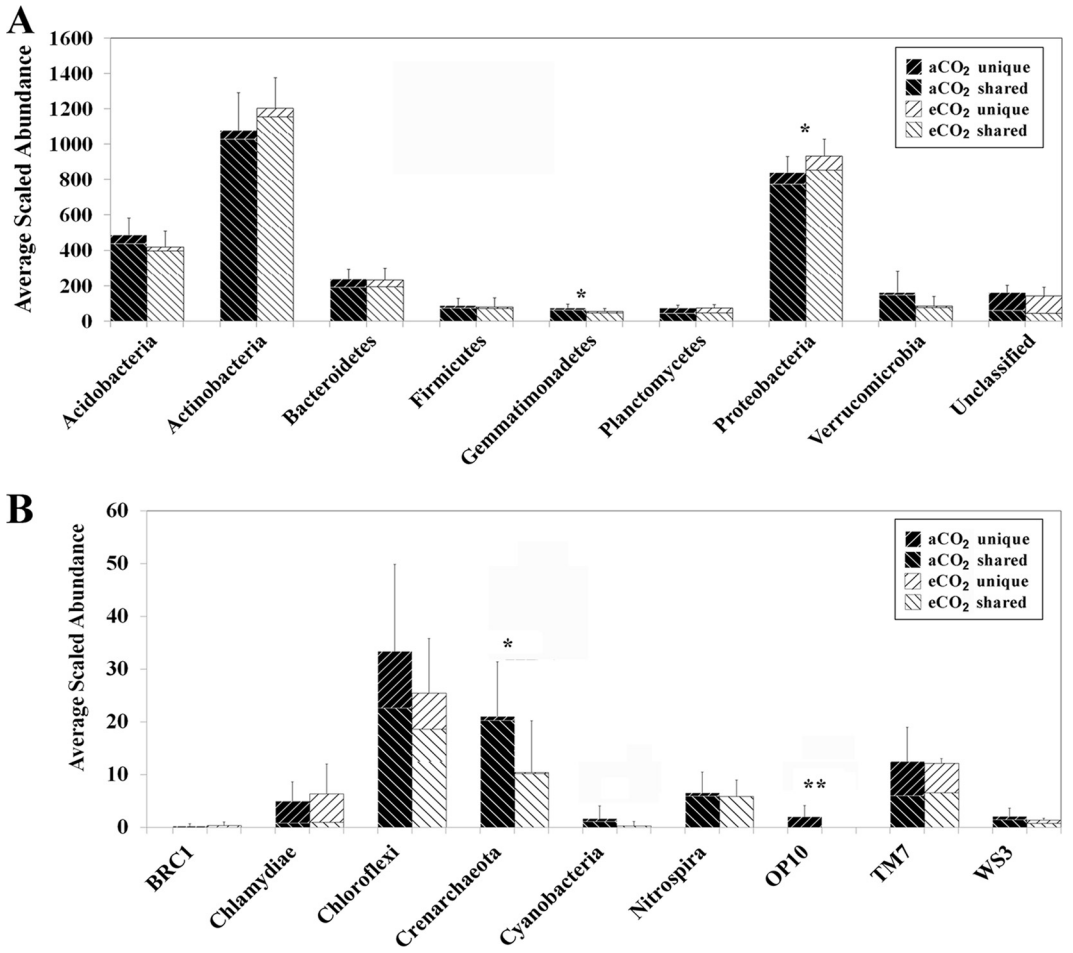

FIG 2 Averages of scaled relative abundances of detected OTUs at the phylum level for both aCO_2 and eCO_2 samples. Data are presented as means ± standard errors, with 12 samples for each CO_2 condition. Generally, each bar has two portions: the top part is for unique OTUs detected only at aCO_2 or eCO_2 and the bottom part for shared OTUs detected under both aCO_2 and eCO_2 conditions. *, $P < 0.05$; **, $P < 0.01$.

populations at the phylum or lower levels. Based on relative abundances of all OTUs in a given phylotype with at least six OTUs detected, significantly changed populations were identified by response ratio (19). At the phylum level, four phyla, including one archaeal phylum (*Crenarchaeota*) and three bacterial phyla (*Proteobacteria*, *Gemmatimonadetes*, and DP10), showed significant ($P < 0.05$) changes in their relative abundances, but other phyla, including *Actinobacteria*, the most abundant phylum, did not show significant changes in relative abundances at eCO_2 (Fig. 3). A further examination of those significantly changed phyla showed that those changes occurred in some specific microbial populations at the class or lower levels. In *Proteobacteria*, the relative abundances of some orders, such as *Caulobacterales* of *Alphaproteobacteria*, *Myxococcales* of *Deltaproteobacteria*, and *Xanthomonadales* of *Gammaproteobacteria*, significantly ($P < 0.05$) increased at eCO_2 (see Fig. S3 in the supplemental material). Also, although significant changes were not seen at eCO_2 for the most abundant phyla at the phylum level, such significances were detected at class or lower taxonomic levels. For example, in *Acidobacteria*, significant ($P < 0.05$) changes were observed in the relative abundances in the classes of Gp1, Gp2, and Gp3 but not in other classes (see Fig. S4A in the supplemental material); in *Verrucomicrobia*, all significant ($P < 0.05$) changes appeared to be decreased in *Spartobacteria* but not in other classes (see Fig. S4B in the supplemental material); in *Firmicutes*, the relative abundances of unclassified phylotypes were significantly ($P < 0.05$) decreased (see Fig. S4C in the supplemental material). These results indicated that eCO_2 might differentially affect some specific microbial populations at different taxonomic levels such as phylum, class, and order, with decreased or increased relative abundances at eCO_2. Recently, a study using a comprehensive functional gene array, GeoChip 3.0 (12), demonstrated that the functional composition and structure of soil microbial communities were significantly altered at eCO_2 in BioCON, which may have been due to eCO_2-induced shifts in microbial populations. However, the linkage between taxonomical populations and their functions of soil microbial communities needs further investigations.

To link the microbial community structure with soil and plant properties, Mantel tests were performed. First, using the BioENV procedure (9), four plant variables, including the belowground carbon percentage (BPC), aboveground carbon percentage (APC), aboveground total biomass (ATB) and total biomass (TB), and four soil variables, including the proportion of soil moisture at the depth of 0 to 17 cm (PSM0–17), the percentage of C at the

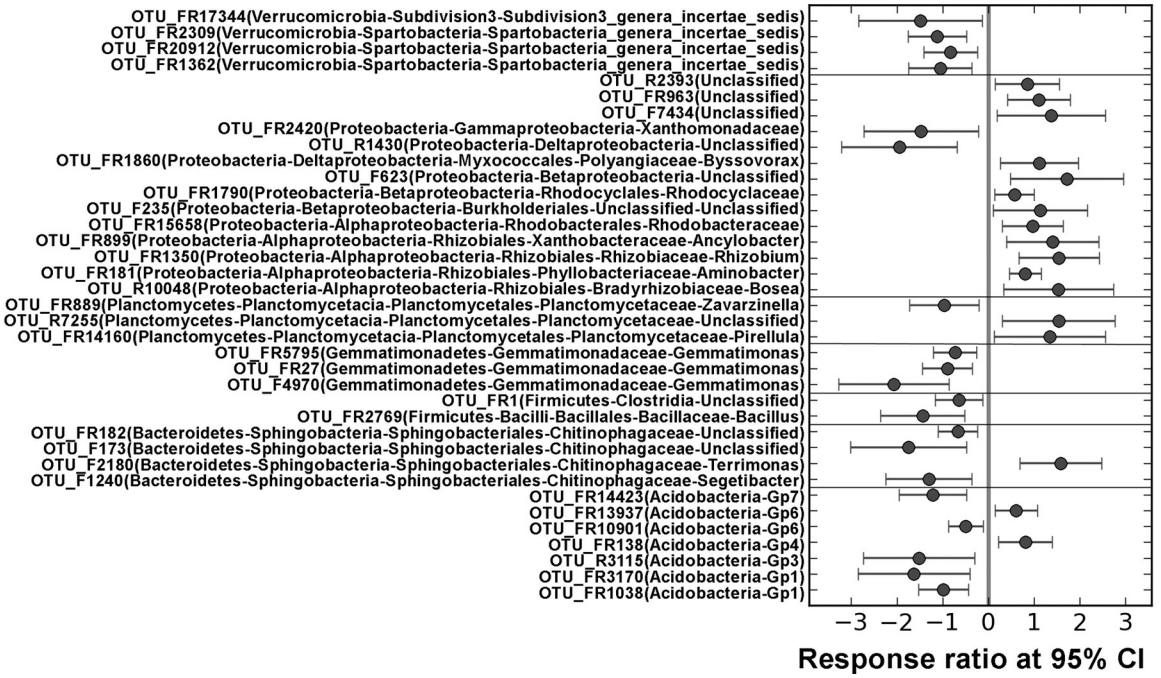

FIG 3 Significantly changed OTUs in the phyla of *Acidobacteria*, *Bacteroidetes*, *Firmicutes*, *Gemmatimonadetes*, *Planctomycetes*, *Proteobacteria*, *Verrucomicrobia*, and unclassified phylotypes at elevated CO_2 determined using the response ratio method at a 95% confidence interval.

depth of 0 to 10 cm (C0–10), the percentage of N at the depth of 0 to 10 cm (N0–10), and net N mineralization, were selected for partial Mantel tests (see Table S6 in the supplemental material). Second, partial Mantel tests were performed to correlate the microbial community measured by the relative abundances of all detected 3,500 OTUs with the selected sets of plant and soil variables described above. Although the microbial community was not significantly ($P > 0.05$) correlated with plant variables or soil variables (see Table S6 in the supplemental material) at the community level, significant correlations were observed with specific microbial populations at different taxonomic levels (phylum, class, order, family, and genus). At the phylum level, five phyla (*Firmicutes*, *Proteobacteria*, *Chlamydiae*, *Gemmatimonadetes*, and *Nitrospirae*) were significantly ($P < 0.05$) correlated with the selected plant properties (see Table S6 in the supplemental material). At the class level, nine classes were significantly ($P < 0.05$) correlated with soil or plant characteristics. *Bacilli*, *Clostridia*, *Alphaproteobacteria*, *Chlamydiae*, *Nitrospirae*, and an unclassified phylotype from the TM7 phylum were significantly ($P < 0.05$) correlated with the selected plant variables, subdivision 3 of *Verrucomicrobiae* ($P = 0.05$) with soil variables, and *Gammaproteobacteria* ($P < 0.05$) with both soil and plant variables (Table 2). Similarly, significant ($P < 0.05$) correlations between the microbial community and the selected plant and/or soil properties were detected in 12 orders (see Table S7 in the supplemental material), 23 families (see Table S8 in the supplemental material), and 48 genera (see Table S9 in the supplemental material). In addition, many unclassified phylotypes were significantly ($P < 0.05$) correlated with the selected soil or/and plant variables, suggesting that soil and plant factors may also shape taxonomically uncharacterized microbial communities (Table 2; see also Table S6, S7, S8, and S9 in the supplemental material). The results presented above suggest that the variables selected greatly influenced the taxonomic composition and structure of microbial communities in this grassland ecosystem.

In summary, this study used pyrosequencing technologies to examine the taxonomical diversity, composition, and structure of soil microbial communities in a grassland ecosystem and their responses to eCO_2. The microbial community composition and structure significantly shifted at eCO_2, potentially due to differen-

TABLE 2 Partial Mantel analysis of the relationship between the relative abundance of class and soil or plant properties[a]

Phylum	Class	Soil,[b] partial plant[c]		Plant,[c] partial soil[b]	
		r	*P*	*r*	*P*
All detected OTUs		−0.018	0.508	0.266	0.065
Firmicutes	*Bacilli*	0.184	0.118	0.379	0.020
	Clostridia	0.040	0.380	0.316	0.014
Proteobacteria	*Alphaproteobacteria*	0.137	0.139	0.300	0.012
	Betaproteobacteria	0.220	0.077	0.470	0.003
	Gammaproteobacteria	0.317	0.023	0.353	0.021
TM7	Unclassified	−0.065	0.621	0.415	0.010
Chlamydiae	*Chlamydiae*	0.192	0.157	0.356	0.026
Nitrospira	*Nitrospira*	−0.033	0.520	0.404	0.013
Verrucomicrobia	Subdivision 3	0.289	0.049	−0.193	0.922

[a] Only significantly ($P < 0.05$) changed phytotypes are shown.
[b] Selected soil variables included proportion soil moisture at the depth of 0 to 17 cm (PSM0 to–17), percent C at the depth of 0 to 10 cm (C0–10), percent N at the depth of 0 to 10 cm (N0–10), and net N mineralization.
[c] Selected plant variables included below ground carbon percent (BPC), above ground carbon percent (APC), aboveground total biomass (ATB), and total biomass (TB).

tial responses of specific microbial populations to eCO_2. Plant and soil properties, such as plant biomass, soil moisture, and soil C and N contents, may greatly shape the microbial community composition and structure and regulate ecosystem functioning in this grassland.

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