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Fungal Community Responses to Past and Future Atmospheric CO_2 Differ by Soil Type

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Soils sequester and release substantial atmospheric carbon, but the contribution of fungal communities to soil carbon balance under rising CO_2 is not well understood. Soil properties likely mediate these fungal responses but are rarely explored in CO_2 experiments. We studied soil fungal communities in a grassland ecosystem exposed to a preindustrial-to-future CO_2 gradient (250 to 500 ppm) in a black clay soil and a sandy loam soil. Sanger sequencing and pyrosequencing of the rRNA gene cluster revealed that fungal community composition and its response to CO_2 differed significantly between soils. Fungal species richness and relative abundance of Chytridiomycota (chytrids) increased linearly with CO_2 in the black clay ($P < 0.04$, $R^2 > 0.7$), whereas the relative abundance of Glomeromycota (arbuscular mycorrhizal fungi) increased linearly with elevated CO_2 in the sandy loam ($P = 0.02$, $R^2 = 0.63$). Across both soils, decomposition rate was positively correlated with chytrid relative abundance ($r = 0.57$) and, in the black clay soil, fungal species richness. Decomposition rate was more strongly correlated with microbial biomass ($r = 0.88$) than with fungal variables. Increased labile carbon availability with elevated CO_2 may explain the greater fungal species richness and Chytridiomycota abundance in the black clay soil, whereas increased phosphorus limitation may explain the increase in Glomeromycota at elevated CO_2 in the sandy loam. Our results demonstrate that soil type plays a key role in soil fungal responses to rising atmospheric CO_2.

As atmospheric CO_2 concentration increases, the soil fungal community will likely change in composition and function. As decomposers and plant symbionts, fungi can regulate the rate of ecosystem carbon (C) cycling. Fungal community composition is particularly important to this rate, because decomposition of complex soil carbon requires a greater diversity of enzymes than any single fungal species can supply (1, 2).

Fungal diversity responses to elevated CO_2 are not well understood, however. One approach for examining fungal species diversity uses a percent similarity cutoff for an evolutionarily conserved gene, creating an operational taxonomic unit (OTU). OTU richness of a fungal cellulose-degrading enzyme (cellobiohydrolase I) increased in an aspen plantation and in the root zone of creosote after 10 years of CO_2 enrichment but showed no response to elevated CO_2 in scrub oak/palmetto, loblolly pine, or marsh ecosystems (3). Cellobiohydrolase I is an extracellular enzyme used in decomposing soil organic matter and is widespread among ascomycete and basidiomycete fungi (3, 4). Ectomycorrhizal fungi have also shown a mixed response to CO_2; although ectomycorrhizal OTU richness and Shannon diversity were unaffected by 6 years of CO_2 enrichment in a pine forest, relative abundances of dominant taxa differed between ambient and elevated CO_2 (5).

The abundance of mycorrhizal fungi will likely increase as atmospheric CO_2 rises. As plants fix and allocate more atmospheric C to roots, they will likely require more soil nitrogen (N) and phosphorus (P) to support increased growth. Because mycorrhizal fungi are important for acquiring soil N and P—the two nutrients that most often limit terrestrial net primary productivity (NPP)—plants may invest more in mycorrhizal associations under elevated CO_2. For example, arbuscular and ectomycorrhizal abundance increased by ~47% on average across 14 elevated CO_2 field experiments, as assessed by the percentage of root length colonized (6). Arbuscular mycorrhizae are best known for transferring soil P to plants (7) but can also transfer soil N (8, 9), whereas ectomycorrhizae are well known to supply plants with N and P and can be important in transporting other nutrients, such as K and water (10). Increased root C availability with elevated CO_2 may also increase colonization by parasitic mycorrhizae, as in a study with forest ectomycorrhizae (5).

A survey of elevated CO_2 field experiments (Table 1) suggests a conceptual model for how elevated CO_2 might affect the soil fungal community in a terrestrial ecosystem. Direct effects of CO_2 on soil fungi are unlikely, as soil CO_2 concentrations are much higher (2,000 to 38,000 ppm) than either present-day atmospheric CO_2 or concentrations predicted for the end of this century (11). Instead, most studies describe increased plant productivity, including root biomass, as the likeliest driver of fungal responses. Increased root litter and exudation are hypothesized to affect fungi by increasing or chemically changing C substrates entering soil (3, 12, 13). This may lead to faster soil C cycling, as increased substrate availability can stimulate fungal enzyme production, including cellulolytic enzymes (12). Increased root biomass could also mean additional sites for mycorrhizal and rhizosphere fungal colonization, and greater root C could allow more species to persist, increasing fungal diversity (3, 5, 14, 15).

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TABLE 1 Soil fungal responses to elevated CO_2 in field experiments and hypothesized mechanisms

Ecosystem	Fungal response to elevated CO_2	Hypothesized mechanisms	Reference(s)
Desert shrub, aspen, loblolly pine, scrub oak, tidal marsh	Cellulolytic enzyme diversity increased or showed no response, depending on ecosystem	Higher plant cellulose availability with elevated CO_2 stimulates cellulose-degrading fungi; fungal community difference between ecosystems reflects differences in soil properties	3
Pine forest	Relative abundance of ectomycorrhizal fungi, but not species richness, changed with elevated CO_2; EM communities grew more dissimilar with elevated CO_2	Greater fine root biomass with elevated CO_2 increased the no. of colonizable sites for EM fungi; also, greater resource availability with elevated CO_2 allowed new species to invade	5
Chaparral	AM fungi shifted from *Glomus* dominance at low CO_2 to *Scutellospora* and *Acaulospora* dominance at high CO_2; AM hyphal length in bulk soil did not increase with elevated CO_2	Decreased N availability at elevated CO_2 may favor low-N-adapted AM fungal taxa; Collembola increased with elevated CO_2 and may have consumed the AM fungal hyphae, reducing apparent CO_2 response in their length	18, 54
Aspen	No effect of elevated CO_2 on fungal abundance in phospholipid fatty acid profiles in low-N or high-N soil	In this young, developing ecosystem, the CO_2-influenced effect of root inputs on microbial communities appeared negligible compared to the effect of native soil organic matter	13
Grassland	AM fungal hyphae increased linearly across a natural CO_2 gradient	Resource balance model: greater aboveground resources (CO_2) led plants to allocate more carbon to acquiring belowground resources, through increased mycorrhizal symbiosis	53
Grassland	No effect of elevated CO_2 on soil microbial community composition or biomass	Plant productivity response to elevated CO_2 was reduced compared to that in other studies, resulting in no CO_2 response in soil microbial communities	71
Grassland	Elevated CO_2 increased microbial biomass and enzyme activities; elevated CO_2 stimulated bacterial biomass but decreased fungal biomass	Increased root production and rhizodeposition with elevated CO_2 stimulated microbial biomass and activity; effects of plant species and soil physiochemical properties likely mediate microbial community responses to CO_2	12

In this study, we examine how the fungal community in a Texas grassland responds to a preindustrial-to-future (250 to 500 ppm) CO_2 gradient, and we investigate how soil properties affect fungal community and decomposition responses to CO_2. Soil microbial community structure and function can differ due to soil physical and chemical properties, such as organic matter content, mineral nutrients, pH, texture, and cation exchange capacity (16–18). For example, the arbuscular mycorrhizal (AM) fungal genus *Glomus* has been associated with plants in clay-rich soil, whereas the genus *Gigaspora* is more commonly associated with plants in sandy soil in several ecosystems (19–21). Several correlated properties that increase with clay content (soil N, P, and organic C) may be driving the prevalence of *Gigaspora* in sandy soil (19–21). Few elevated CO_2 studies have included more than one soil type, however (22–24). Our study addresses this knowledge gap by examining AM fungal and total fungal community responses to CO_2 on two soil types, a black clay and a sandy loam, both represented along the experimental CO_2 gradient.

We tested three predictions of CO_2 effects on fungal communities. (i) We predicted that increased rhizodeposition with elevated CO_2 would increase fungal species richness in the black clay but not the sandy loam soil. As a corollary, we predicted that decomposition rates would increase with elevated CO_2 in the black clay. This is based on our observation that labile C pools (easily decomposed root litter and exudates) increased more with elevated CO_2 in the black clay soil than in the sandy loam soil (A. Procter, R. Gill, P. A. Fay, H. W. Polley, and R. B. Jackson, unpublished data). If this increased labile C relieves C limitation and allows additional saprophytic fungal species to colonize, the decomposition rate could increase. (ii) We predicted that the AM fungal community in the black clay soil would shift from *Glomus* dominance at subambient CO_2 to *Scutellospora* dominance at elevated CO_2. These taxa have different nitrogen demands (18), and two lines of evidence suggest elevated CO_2 increased soil N limitation in our experiment. First, inorganic N availability in the black clay soil declined with increasing CO_2 in the year we sampled fungal communities (25, 26). Second, recalcitrant organic N-degrading enzyme activity (likely from saprophytic fungi and bacteria) increased with elevated CO_2 in the black clay (25, 27, 28). N limitation in the black clay could shift the AM fungal community toward low-N-adapted *Scutellospora* species. (iii) Finally, we predicted that mycorrhizal abundance would increase with CO_2 treatment, particularly in the sandy loam. Aboveground productivity and soil alkaline phosphatase activity increased linearly with CO_2 in the sandy loam (25, 26), suggesting increased soil P limitation. Grass tiller P also decreased with CO_2 in the sandy loam and black clay, suggesting P limitation in both soils (29). Plants experiencing CO_2-fertilized growth may relieve phosphorus limitation by promoting mycorrhizal associations.

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TABLE 2 Soil properties in the CO_2 gradient experiment[a]

Property	Value		P value
	Sandy loam (Bastsil series)	Black clay (Houston series)	
ANPP (g/m^2)	583	537	0.44
Organic C (%)	0.8	2.7	<0.0001
Total C (%)	0.9	5.5	<0.0001
Total N (%)	0.08	0.22	<0.0001
Organic C/total N	10.8	12.2	0.0016
Sand (%)	60–72	9–11	
Silt (%)	20–26	36–40	
Clay (%)	7–14	49–55	
pH (in H_2O)	8.09	7.98	0.10

[a] *P* values are from *t* tests of soil type means; data are from soil samples used in this study. Aboveground net primary production (ANPP) is averaged across the CO_2 gradient in 2009. Organic C was measured by combustion at 600°C; total C and N by combustion at 1,050°C. Soil textures are for the upper 50 cm of soil (Table 2 in Fay et al. [24]). Soil pH was measured in July 2009 soil samples and averaged across the CO_2 gradient.

MATERIALS AND METHODS

Study system. The research was conducted at a grassland CO_2-enrichment facility at the USDA-ARS Grassland, Soil and Water Research Laboratory, in Temple, TX (24, 25). Air was blown through two elongated chambers covered in clear plastic, and plant photosynthesis progressively depleted the air of CO_2. CO_2-enriched air was injected in one chamber to create a continuous gradient of elevated CO_2 (500 to 380 ppm); ambient air was injected into the other chamber to create a continuous gradient of subambient CO_2 (380 to 250 ppm). Computer control of fan speed maintained each gradient despite varying photosynthesis. At night, the airflow reversed direction, and respiration from plants and soil maintained the CO_2 gradient. CO_2 concentrations along the gradient were 120 ppm higher at night, ranging from 370 to 500 ppm and 500 to 620 ppm in the subambient and elevated chambers, respectively.

Each elongated CO_2 chamber was divided into 10 consecutive 5-m-long compartments. We installed four steel boxes containing intact soil monoliths (each 1 m wide and long and 1.5 m deep) beneath each of the 20 5-m-long compartments. Monoliths were placed in open-topped steel bins buried to a 1.3-m depth along each chamber. Three soil types of contrasting physical and hydrological properties were randomly placed throughout the CO_2 gradient, such that each 5-m-long compartment contained two monoliths of each of two soil types. For this study, we focused on the two soil types which differed most in texture: a sandy loam Alfisol (Bastsil series, formerly Bastrop series) with 15% clay and 60 to 73% sand in the upper 50 cm and a black clay Vertisol (Houston series) with 55% clay in the upper 50 cm (Table 2) (24). The black clay has higher organic C, total C, total N, and organic C/N than the sandy loam ($P < 0.05$), but the two soils have similar levels of plant productivity. The sandy loam is slightly more basic than the black clay ($P = 0.10$).

Prior to monolith excavation and installation in the CO_2 gradient, *Buchloe dactyloides* (buffalo grass) and *Panicum obtusum* (vine mesquite) were dominant in the black clay soil, and *Sorghum halepense* (Johnson grass) and *Schizachyrium scoparium* (little bluestem) were dominant on the sandy loam soil. All vegetation was herbicided in preparation for planting. In 2003, a mixture of perennial grasses and forbs was transplanted into the monoliths: C_4 grasses *Sorghastrum nutans*, *Bouteloua curtipendula*, *Schizachyrium scoparium*, *Tridens albescens*; C_3 forbs *Salvia azurea*, *Solidago canadensis*; and the legume *Desmanthus illinoensis*. The obligately mycorrhizal grasses *S. nutans* and *S. scoparium* (30, 31) are dominant species in the CO_2 gradient plant community (29). The ecosystem represents Texas blackland prairie, found near the experimental site and known for the black color of its namesake soil, the Houston black clay. CO_2 treatment began in 2006 and extends from April to October, a time when photosynthesis is sufficient to maintain the CO_2 gradient.

Soil sampling and DNA extraction. We sampled soil in the third and fourth growing seasons of CO_2 treatment (November 2008 and July 2009) to study the effects of CO_2 and soil type on fungal communities. In a November 2008 study, we took sandy loam soil cores at two CO_2 concentrations: 300 ppm and 475 ppm. The following July (2009), soil cores were taken from the black clay soil at six CO_2 concentrations between 271 to 500 ppm and from the sandy loam at eight CO_2 concentrations between 290 to 480 ppm for DNA extraction. We also sampled soil in November 2009 to study how decomposition rates responded to the CO_2 gradient: 10 CO_2 concentrations for the black clay and eight CO_2 concentrations for the sandy loam. Decomposition measurements are described below. For soils collected for DNA extraction, we composited four 1-cm-diameter by 3-cm-deep cores per soil type within a gradient compartment as one sample representing one CO_2 concentration (see Fig. S1 in the supplemental material). The July 2009 samples represent a soil composite for the plant community at each CO_2 concentration, while the November 2008 samples were collected near the obligately mycorrhizal grass *Sorghastrum nutans*, originally as part of a growth chamber experiment (Procter et al., unpublished). Soil samples were stored at −80°C before analysis. DNA was extracted from soil samples using the PowerSoil DNA kit (MO BIO Laboratories, Carlsbad, CA, USA). We performed three replicate DNA extractions, each using 0.25 g soil, on each composited soil sample at a given CO_2 level, for a total of 48 extractions (8 black clay samples and 6 sandy loam samples from July 2009, and 2 sandy loam samples from November 2008, each extracted three times). DNA was eluted in autoclaved Nanopure water (Barnstead International, Dubuque, IA) and stored at −20°C.

Pyrosequencing of the fungal community. We performed 454 pyrosequencing of the total fungal community for both November 2008 and July 2009 samples. We used the fungus-specific primer ITS1f (32) and the general eukaryotic primer ITS2 (33) to amplify the first internal transcribed spacer (ITS) region of the rRNA gene. Primers included a unique 10-nucleotide (nt) barcode for each sample (see Table S1 in the supplemental material). PCRs had initial denaturation at 95°C for 15 min, 10 touchdown cycles of 94°C for 45 s, 60°C for 30 s (declining 1°C with each cycle), and 72°C for 45 s, followed by 25 cycles of 94°C for 45 s, 50°C for 30 s, and 72°C for 45 s, with a final extension at 72°C for 10 min. The 25-μl PCR mixture contained 3 μl template DNA (1 μl from each of 3 replicate extracts per soil sample) and was otherwise identical to the AM fungal PCRs mentioned above. The amplicons, about 350 to 400 bp long, were sequenced unidirectionally from the ITS1f primer on a Roche 454 GS-FLX system using Titanium and LibL chemistry (454 Life Sciences, Branford, CT).

ITS sequence editing and diversity analysis. We used the bioinformatics software Mothur to trim and analyze sequences (34). After we screened for sequence quality, removing primer sequences and eliminating sequences shorter than 180 nt, the data set contained 20,371 sequences, with each of the 16 composited soil samples represented by about 1,000 sequences. The average sequence length was 223 nt. After producing a distance matrix based on pairwise alignment of sequences, we clustered sequences into operational taxonomic units (OTUs) at 97% similarity using average-neighbor linkage. This similarity cutoff is often used to define fungal species based on the ITS region (35–37). To compare relative species richness, communities were rarefied to 800 sequences, which was the smallest number of sequences obtained among the 16 samples (see Fig. S2 in the supplemental material). A similar approach for comparing relative species richness was used by Buee et al. (36), Jumpponen et al. (38), and Rousk et al. (17). Further diversity analyses for our samples were performed in PC-ORD 6 (MJM Software, Gleneden Beach, OR). We used nonmetric multidimensional scaling (NMS) and a Mantel test to determine the effects of CO_2 and soil type on community composition. Community distances for the NMS and Mantel tests were calculated using Bray-Curtis dissimilarity of OTU relative abundances. The Mantel test measured correlation between community distance and soil type distance matrices, coded as 0 if samples shared the same soil and 1 if they did not.

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ITS sequence taxonomies were analyzed using two approaches. First, we used a BLAST-based classifier program described in reference 37, referencing a database of >600,000 ITS sequences downloaded from NCBI. The top match (with the lowest E value) was used for identification, and sequences with <75% maximum identity to any database sequence were left unclassified. The classified sequences were grouped by taxonomic rank: phylum, class, order, family, and genus. These abundances were tabulated to represent community composition and calculate relative abundances of taxa. In the second approach, a representative sequence for each OTU was chosen based on the shortest distance from all other sequences in the OTU. Each OTU representative was identified with a Bayesian classifier implemented in Mothur, referencing the UNITE database (version 6, dynamic species cutoff, http://www.mothur.org/wiki/UNITE_ITS_database).

Cloning and sequencing of AM fungi. A nested PCR approach, modified from reference 39, was used to selectively amplify ribosomal DNA from communities of arbuscular mycorrhizal fungi in the July 2009 samples. The first PCR amplification used the general eukaryotic primers LR1 and NDL22 (40), and its PCR product was used as the template for a second PCR amplification with the AM fungus-specific large ribosomal subunit primers LR1 and FLR4 (41). The final product was ~700 bp. The first PCR had initial denaturation at 95°C for 15 min, 30 cycles of 94°C for 45 s, 60°C for 45 s, and 72°C for 45 s, followed by a final extension at 72°C for 5 min. Each 25-μl reaction mixture contained 3 μl template DNA (1 μl from each of 3 replicate extracts per soil sample), 1 μl bovine serum albumin, 0.5 μl Apex Hot Start DNA polymerase, 1 μl $MgCl_2$, 4 μl deoxynucleoside triphosphates (dNTPs), 1.25 μl each primer, 2.5 μl 10× Apex buffer I, and 10.5 μl water. Stock concentrations were as follows: 10 mg/ml bovine serum albumin (BSA), 5 units/μl DNA polymerase, 50 mM $MgCl_2$, 1 mM dNTPs, 10 μM each primer. For the second PCR, the first PCR product was diluted 1:1,000 (black clay soil) or 1:100 (sandy loam soil) and used as the template. A more concentrated template was used for the sandy loam soil, because in initial tests, more dilute template yielded less product (or no product) compared to that of the black clay soil DNA. This suggests that AM fungal DNA was less abundant in the sandy soil or that the second-round primers amplified less efficiently in DNA extracts from the sandy loam. The second PCR was identical to the first, except it used 26 cycles rather than 30, and each 25-μl reaction mixture contained 6 μl diluted template rather than 3 μl.

The second PCR product was cloned in *Escherichia coli* using a TOPO-TA cloning kit (Invitrogen, Carlsbad, CA). At least 50 clones per sample were amplified using a colony PCR protocol (35). Colony PCR products were purified using the ExoSAP-IT kit (Affymetrix, Santa Clara, CA). Cycle sequencing was performed using the M13F forward primer and BigDye v3.1 chemistry (Life Technologies, Carlsbad, CA).

AM fungal sequence editing and diversity analysis. AM fungal sequences were edited using Sequencher 4.9 (Gene Codes, Ann Arbor, MI). All sequences were BLAST searched against the NCBI database, and non-*Glomeromycota* sequences were removed (<5% of all sequences) for a final data set of 50 sequences per sample. A multiple-sequence alignment was constructed and manually edited in Mesquite (42), using Clustal W2 (43). The edited aligned sequences were clustered in Mothur (34) using a 95% similarity, furthest-neighbor definition for OTUs. This 95% similarity cutoff estimates species richness more conservatively than 97% and better matches the OTUs defined in reference 39, which used a similar nested PCR approach to sequence AM fungi using large ribosomal subunit DNA from soil samples. Sequences representative of each OTU were used to construct a neighbor-joining tree with MEGA 5 software (44). The tree allowed us to classify OTUs by the revised AM fungal taxonomy (45, 46). In the revised taxonomy, the former genus *Glomus* has been reclassified as two families containing the five genera *Rhizophagus*, *Sclerocystis*, *Funneliformis*, *Claroideoglomus*, and *Glomus*. OTU diversity analyses for our samples were performed in PC-ORD 6 (MJM Software, Gleneden Beach, OR), using methods described above for the total fungal community.

Carbon mineralization. At the end of the fourth growing season of CO_2 treatment (9 November 2009), soils from the CO_2 gradient were cored to a 15-cm depth, air dried, and passed through a 2-mm sieve to remove roots. Cores were air dried so that they could be moistened to 60% field capacity for long-term incubation. Incubation at constant moisture and temperature in the dark isolated the effect of native microbial biomass and available carbon on decomposition rates. Soils were kept at 21°C in 1-pint (473-ml) canning jars with septa added to their lids (47, 48). Within each jar, a plastic cup contained 40 g (dry mass) soil moistened to 60% field capacity. Field capacity differed by soil type and was determined using Bastsil (sandy loam) and Houston (black clay) series soils from the respective source locations of soils in the CO_2 gradient (49). Before measuring carbon mineralization rate, soils were incubated for 12 days to correct for artifacts induced by drying and rewetting the soils (48). Carbon mineralization (C_{min}) was measured using an LI-6200 portable photosynthesis system and an LI-6250 CO_2 analyzer (LI-COR, Lincoln, NE). C_{min} rate was calculated from the rate of CO_2 buildup in jars. Jars were capped and CO_2 was measured immediately and again after 2 and 4 h to determine the average rate of CO_2 concentration buildup. Jar headspace air was sampled using a 1-ml glass syringe.

Soil respiration. Soil respiration was measured as a field index of belowground carbon bioavailability and microbial activity, to complement the laboratory measure of these variables (C_{min} rate). It is roughly 50% root respiration and 50% microbial respiration, though these contributions can vary (50, 51). Soil respiration has been measured monthly (May to October) since the start of CO_2 gradient operation in 2006. Soil respiration is measured instantaneously through vegetation-free soil collars using an LI-COR 6400 infrared gas analyzer (LI-COR, Lincoln, NE).

Nucleotide sequence accession numbers. The fungal ITS sequences were deposited in GenBank under BioProject no. PRJNA205400, and the AM fungal LSU sequences were deposited under accession numbers KC410873 to KC411472.

RESULTS

Fungal communities. Fungal OTU richness increased linearly with CO_2 concentration in the black clay soil but did not change in the sandy loam ($R^2 = 0.71$ for the black clay, $P = 0.035$) (Fig. 1). In the black clay, fungal communities contained on average 23 more OTUs at elevated CO_2 than at ambient CO_2, while communities at subambient CO_2 contained on average 6 fewer OTUs than at ambient CO_2. Mean OTU richness did not differ by soil type (*t* test, $P = 0.93$). Using universal fungal primers, pyrosequencing of the six black clay soil samples and eight sandy loam soil samples produced 20,371 ITS sequences, which grouped into 792 OTUs using a 97% similarity, average-neighbor definition. Rarefaction curves made from each sample did not plateau, indicating additional unsequenced diversity (see Fig. S2 in the supplemental material); therefore, we compared the relative species OTU of communities rarefied to 800 sequences.

Nonmetric multidimensional scaling (NMS) axis 1 explained most (75%) of the variation in fungal community composition and separated communities by soil type (Fig. 2A). NMS explored how fungal communities differed both in species richness and relative abundance. NMS analysis produced a 2-axis solution with a final stress of 5.709; this low stress suggests consistency between distances on the NMS plot and differences in community composition. Fungal community difference was significantly correlated with soil type difference (Mantel $r = 0.82$, $P = 0.001$).

Elevated CO_2 altered fungal community structure in the black clay soil. For communities sampled in July 2009, CO_2 explained 71% of the variation in NMS axis 2 scores in the black clay but was not associated with axis 2 scores in the sandy loam, even after one outlier was removed (Fig. 2B). Soil type explained nearly all vari-

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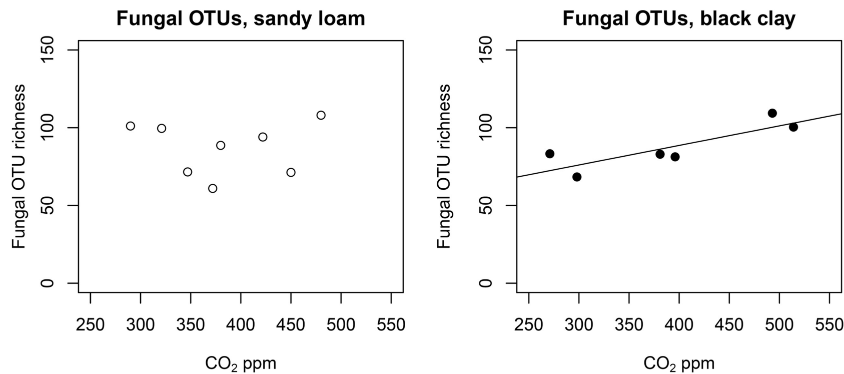

FIG 1 OTU richness of total fungal communities in sandy loam and black clay soils. Fungal OTUs are defined at 97% similarity (roughly species level). Communities were rarefied to 800 sequences to calculate relative OTU richness, which increased linearly with CO_2 in black clay soil ($R^2 = 0.71$, $P = 0.035$).

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ation in axis 1 scores ($R^2 = 0.97$, $P < 0.01$ by linear regression) (Fig. 2A), and axis 1 scores were not correlated with CO_2 in either sandy loam, black clay, or both soil types combined. Therefore, CO_2 effects (axis 2) explained less than 10% of variation in community composition and were distinct from soil type effects (axis 1), which explained nearly 75% of variation in composition. CO_2 changed fungal community structure in the black clay, but soil type effects on community structure were stronger than CO_2 effects.

The relative abundance of dominant fungal OTUs shifted with CO_2 treatment. OTU9, the dominant OTU for total fungal communities, peaked at 380 ppm CO_2 in the black clay (Fig. 3). OTU9 comprised 7 to 54% of black clay fungal libraries but less than 5% of sandy loam libraries. Using a Bayesian classification based on the UNITE database (52), OTU9 matched phylum Basidiomycota, order Tremellales, and genus *Derxomyces*, although the order and genus had low bootstrap support (16% and 10%, respectively) (Table 3). The second most abundant OTU for total fungal communities (OTU3) also matched order Tremellales, but with low bootstrap support at the order and genus levels. OTU3 was nearly absent from black clay samples but comprised up to 16% of sequences in sandy loam samples, peaking at 372 ppm CO_2. Seventy percent of the fungal sequences grouped into the 32 most abundant OTUs. The closest UNITE database matches to these OTUs are mostly phylum Basidiomycota or Ascomycota, although one OTU could not be classified to phylum (OTU14), and one (OTU132) was from a basal fungal lineage (formerly phylum Zygomycota). Of the 792 OTUs composing the total fungal community, nearly half (363) were observed only once. These rarest OTUs have similar phylum-level diversity to the dominant 32 OTUs; most of the rare OTUs were Ascomycetes and Basidiomycetes, 10 matched a basal fungal lineage (formerly Zygomycota), 6 matched Glomeromycota, 6 matched Chytridiomycota, and 11 were unclassified.

Of the 792 total OTUs, the relative abundance of eight OTUs changed linearly with CO_2 concentration (see Table S2 in the supplemental material). Two of these OTUs composed more than 1% of sequences in the data set: OTU10, a basidiomycete, and OTU2, an ascomycete. The four OTUs that responded to CO_2 in black clay soil differed from the four OTUs that responded in the sandy loam soil. OTU279, which increased significantly with CO_2 in the

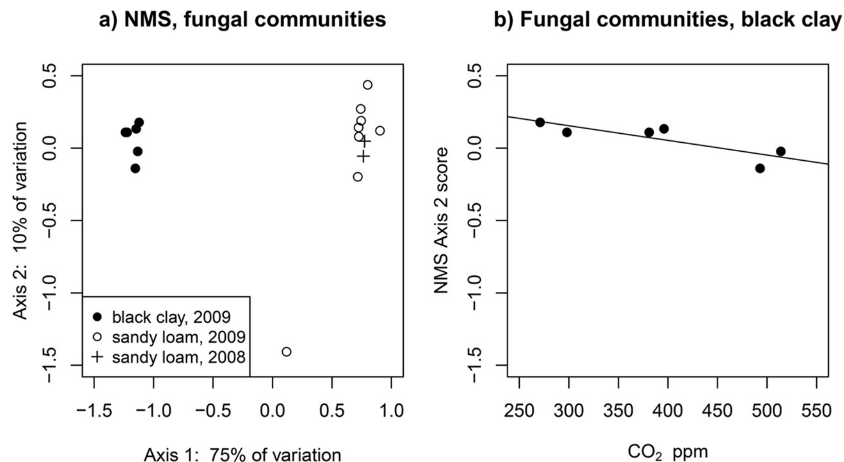

FIG 2 (a) NMS plot of total fungal communities in the CO_2 gradient. The percentage of variation in community composition represented by each axis is shown. Community distances were based on OTU relative abundances. For July 2009 samples, sandy loam soils are shown in white and black clay soils are shown in black. November 2008 samples are from sandy loam only and are shown as plusses (+). (b) CO_2 effect on total fungal community composition, based on the NMS in panel a. NMS axis 2 scores are an index of community composition and decrease linearly with CO_2 concentration in the black clay ($R^2 = 0.71$, $P = 0.04$). Axis 2 scores are not linearly related to CO_2 in the sandy loam, even after one outlier is removed (290 ppm, −1.4 on axis 2).

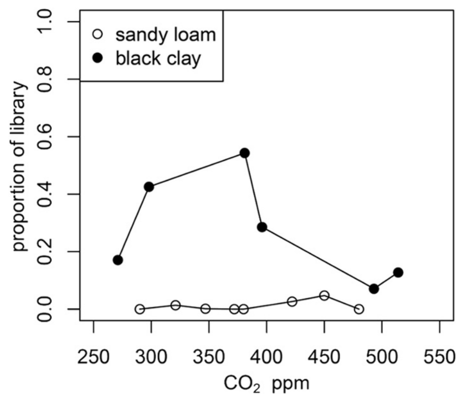

FIG 3 The relative abundance of the dominant OTU from total fungal communities, as a function of CO_2 level. The closest UNITE database match to OTU9 was phylum Basidiomycota, order Tremellales, and possibly genus *Derxomyces*.

sandy loam, matched the AM fungal genus *Entrophospora* (family Acaulosporaceae).

Soil type affected phylum-level responses to CO_2 (Fig. 4). Although the two dominant phyla (Ascomycota and Basidiomycota) did not respond to CO_2, the relative abundance of Glomeromycota (AM fungi) increased linearly with elevated CO_2 in the sandy loam, supporting our prediction that increased P limitation with elevated CO_2 would favor mycorrhizal colonization in the sandy loam. Based on plant tissue [P] and biomass, we calculated that P uptake into aboveground biomass increased with elevated CO_2 in the sandy loam ($R^2 = 0.4$, $P = 0.09$), further suggesting P limitation in this soil (see Table S3 in the supplemental material). Unlike Glomeromycota, Chytridiomycota relative abundance increased linearly with CO_2 in the black clay. A targeted pyrosequencing study using the same soil DNA extracts as in the present study showed increased Chytridiomycota relative abundance at 514 ppm CO_2 in the black clay soil, which supports this finding. In the current analysis, 11,960 sequences that did not receive a phylum-level classification against our database were excluded. On

TABLE 3 Thirty-two most abundant fungal OTUs in the CO_2 gradient experiment (both soil types, 454 pyrosequencing)

Rank	OTU ID	% of total sequences[a]	Cumulative % of total[b]	Phylum[c]	Order[c]	Genus[c]
1	9	12.3	12	Basidiomycota (64)	Tremellales (16)	*Derxomyces* (10)
2	3	5.0	17	Basidiomycota (77)	Tremellales (22)	*Bullera* (12)
3	11	4.2	21	Basidiomycota (51)	Tremellales (17)	*Bullera* (16)
4	1	3.9	25	Basidiomycota (98)	Auriculariales (96)	Unknown Auriculariales (94)
5	59	3.7	29	Basidiomycota (100)	Agaricales (100)	*Stropharia* (63)
6	37	3.3	32	Basidiomycota (100)	Agaricales (100)	Unknown Agaricales (53)
7	78	3.3	36	Basidiomycota (100)	Agaricales (100)	*Conocybe* (100)
8	10	3.1	39	Basidiomycota (62)	Tremellales (16)	*Derxomyces* (1)
9	14	3.1	42	Unknown phylum (42)	Unknown order (42)	Unknown fungi (42)
10	17	2.8	45	Ascomycota (100)	Sordariales (99)	Unknown Sordariales (98)
11	2	2.5	47	Ascomycota (61)	Unknown Archaeorhizomycetes (22)	Unknown Archaeorhizomycetes (22)
12	13	2.2	49	Basidiomycota (70)	Tremellales (21)	*Bullera* (15)
13	7	2.1	51	Ascomycota (100)	Pleosporales (100)	*Alternaria* (100)
14	72	1.8	53	Basidiomycota (100)	Agaricales (100)	Unknown Psathyrellaceae (72)
15	45	1.6	55	Basidiomycota (61)	Unknown Wallemiomycetes (61)	Unknown Wallemiomycetes (61)
16	23	1.4	56	Basidiomycota (59)	Tremellales (18)	*Derxomyces* (6)
17	134	1.3	58	Basidiomycota (98)	Agaricales (94)	*Coprinopsis* (33)
18	28	1.2	59	Basidiomycota (69)	Tremellales (10)	*Derxomyces* (0)
19	60	1.1	60	Ascomycota (92)	Chaetothyriales (73)	*Rhinocladiella* (23)
20	56	1.1	61	Ascomycota (100)	Sordariales (100)	*Podospora* (96)
21	246	1.0	62	Basidiomycota (73)	Tremellales (21)	*Bullera* (19)
22	49	1.0	63	Basidiomycota (100)	Agaricales (100)	*Psathyrella* (98)
23	132	0.8	64	Fungus incertae sedis (formerly Zygomycota) (100)	Mortierellales (100)	*Mortierella* (100)
24	36	0.8	65	Basidiomycota (100)	Auriculariales (82)	Unknown Auriculariales (75)
25	99	0.8	65	Basidiomycota (99)	Agaricales (98)	*Hygrocybe* (96)
26	39	0.8	66	Ascomycota (84)	Helotiales (34)	*Tetracladium* (29)
27	16	0.7	67	Ascomycota (98)	Pleosporales (98)	*Stagonospora* (20)
28	64	0.7	68	Ascomycota (96)	Hypocreales (62)	*Acremonium* (33)
29	51	0.7	68	Ascomycota (67)	Pezizales (24)	*Rhizina* (8)
30	38	0.6	69	Basidiomycota (54)	Tremellales (16)	*Derxomyces* (5)
31	71	0.6	69	Ascomycota (78)	Unknown Dothideomycetes (40)	Unknown Dothideomycetes (40)
32	117	0.6	70	Basidiomycota (49)	Tremellales (11)	*Derxomyces* (8)

[a] Percentage of 20371 ITS sequences represented by each OTU.
[b] Summed percentage of total sequences represented by OTUs through rank 1.
[c] A representative sequence for each OTU was classified using the UNITE database with dynamic species cutoff (http://www.mothur.org/wiki/UNITE_ITS_database). Percent bootstrap support is in parentheses.

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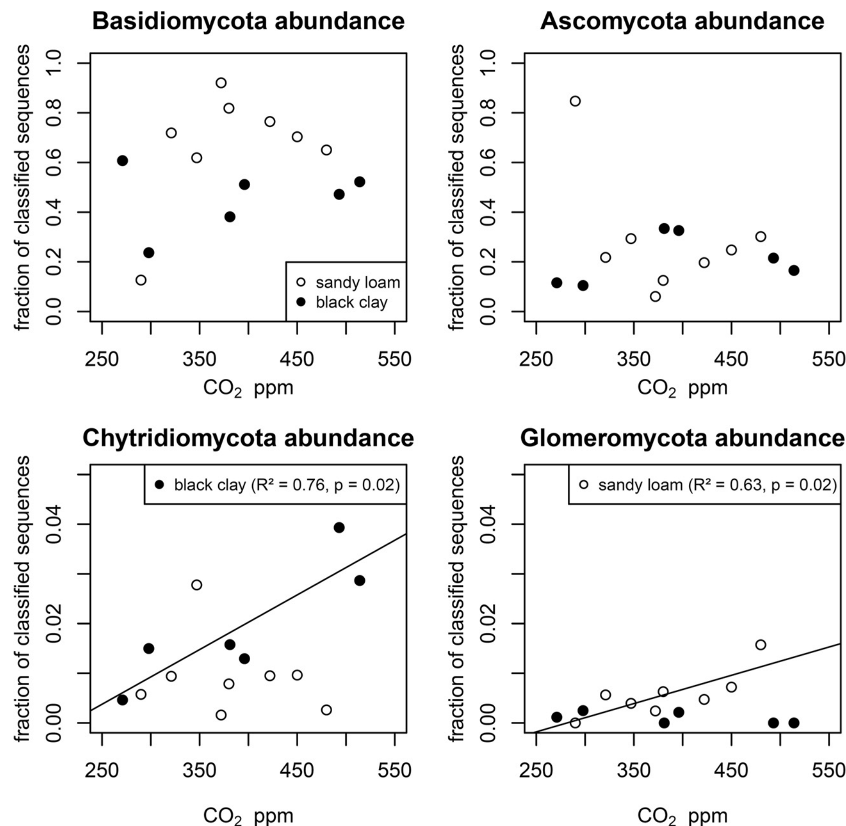

FIG 4 Fungal phylum-level responses to CO_2, graphed by soil type. Chytridiomycota increased linearly with CO_2 in the black clay (dark circles), and Glomeromycota increased linearly with CO_2 in the sandy loam (light circles). Abundances are relative to the fungal community amplified by ITS primers.

average, Chytridiomycota represented 1.3% of classified sequences in each community, and Glomeromycota represented 0.3%.

AM fungal (Glomeromycota) communities. All sequences in the AM fungal data set matched genus *Glomus* in a BLAST search against the NCBI database. However, a phylogeny based on the revised AM fungal taxonomy (see Fig. S3 in the supplemental material) suggested at least four genera in two families (Glomeraceae and Claroideoglomeraceae) (45, 46). The 600 sequences grouped into 104 OTUs using a 95% similarity, furthest-neighbor definition. The 16 most abundant AM fungal OTUs compose 70% of total AM fungal sequences (Table 4). Rarefaction curves made from each sample did not plateau, suggesting further unsequenced diversity (see Fig. S4 in the supplemental material). Similar to total fungal OTU richness, AM fungal OTU richness did not differ by soil type (*t* test, $P = 0.61$) (Fig. 5A). Moreover, AM fungal OTU richness was not affected by CO_2 treatment; linear and quadratic regressions were nonsignificant in either soil, as well as in the pooled data from both soils.

In the AM fungal NMS, communities showed some separation by soil type (Fig. 5B). On the axis explaining the largest portion (41%) of community variation, most sandy loam AM fungal communities had a positive value, while all black clay communities had a negative value. NMS analysis produced a 3-axis solution for AM fungi. AM fungal community difference was marginally correlated with soil type difference (Mantel $r = 0.17$, $P = 0.06$).

The AM fungal data set partially corroborated the total fungal data set. Of the 792 OTUs in the total fungal data set, 12 were classified as Glomeromycota. These AM fungal OTUs were further classified into the genera *Glomus*, *Rhizophagus*, and *Entrophosphora*, with the remainder being unclassified Glomeraceae or unclassified Glomeromycota. Although these results agree with the AM fungal data set in identifying *Glomus* and *Rhizophagus* (family Glomeraceae) species, *Entrophospora* (family Acaulosporaceae) was unique to the total fungal data set.

Environmental predictors of fungal community change. To determine whether CO_2 effects on fungal diversity were mediated by soil pH, we measured pH in the soil samples used for DNA extraction. When data from both soils were combined, soil pH decreased linearly as CO_2 increased ($R^2 = 0.14$, $P = 0.04$), and the sandy loam was slightly more basic than the black clay ($P = 0.009$) (see Fig. S7A in the supplemental material). Total fungal species richness was negatively related to pH ($R^2 = 0.70$, $P = 0.04$) (see Fig. S7B), but only in the black clay. Therefore, increased soil acidity at elevated CO_2 was associated with greater fungal diversity in the black clay. AM fungal species richness was not related to pH ($P = 0.70$).

We tested the prediction that increased labile C availability with elevated CO_2 would increase fungal diversity and, in turn, decomposition rates. Fungal OTU richness increased linearly with soil organic carbon (SOC) lability in the black clay soil ($R^2 = 0.74$, $P = 0.03$) but not the sandy loam (see Fig. S5 in the supplemental material). Although SOC was three times more labile in the sandy loam than the black clay, both soils had a similar range of OTU richness (60 to 110 OTUs). Soil respiration rates across both soils also increased linearly with fungal OTU richness during the

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TABLE 4 Sixteen most abundant AM fungal (phylum Glomeromycota) OTUs in the CO_2 gradient experiment[e]

Rank	OTU ID	% of total sequences[a]	Cumulative % of total[b]	Accession no.	Closest NCBI database match	Query coverage (%)[c]	Maximum similarity (%)[d]
1	4	31	31	FR871375	Uncultured *Glomus*	74	98
2	18	9	40	AB710222	Uncultured *Glomus*	85	100
3	9	5	45	AM397822	Uncultured *Glomus*	72	91
4	2	4	49	EU379997	Uncultured glomeromycete	86	96
5	26	3	51	HM216142	Uncultured *Glomus*	73	92
6	13	2	53	AY394694	Uncultured *Glomus*	78	84
7	60	2	56	JF439185	*Glomus* sp.	85	94
8	10	2	58	JQ650496	Uncultured Glomeromycota	48	100
9	29	2	60	AM397822	Uncultured *Glomus*	79	89
10	27	2	62	FR871345	Uncultured *Glomus*	66	92
11	12	2	63	GQ149201	Uncultured glomeromycete	72	87
12	25	2	65	FR871375	Uncultured *Glomus*	74	98
13	15	1	66	AB640742	Uncultured *Glomus*	87	98
14	38	1	67	GQ149214	Uncultured glomeromycete	78	99
15	11	1	68	AY394694	Uncultured *Glomus*	71	85
16	17	1	70	JQ048859	*Glomus deserticola*	77	88

[a] Percentage of 600 total sequences represented by each OTU.
[b] Summed percentage of total sequences represented by OTUs through rank 1.
[c] Percentage of the query (OTU) sequence that overlaps with the NCBI match.
[d] Percent similarity between the query (OTU) and NCBI match over their region of overlap.
[e] Data generated from Sanger sequencing of both soil types.

month of fungal sampling ($R^2 = 0.46$, $P = 0.01$) (Fig. 6A). Soil respiration rate increased from 7 to 12 μmol $CO_2/m^2/s$ as OTU richness increased from about 70 to 110 (one outlier excluded as noted). Soil respiration is a measure of belowground metabolism that integrates root and microbial respiration. Another measure of decomposition, carbon mineralization (C_{min}) rate, was linearly related to fungal OTU richness (Fig. 6B), but with a positive slope in the black clay ($R^2 = 0.72$, $P = 0.03$) and a negative slope in the sandy loam ($R^2 = 0.43$, $P = 0.08$). In the black clay, C_{min} rate increased from 12 to 18 μg C g^{-1} soil day^{-1} as OTU richness increased from about 70 to 110. In the sandy loam soil, C_{min} rate increased from 10 to 16 μg C g^{-1} soil day^{-1} as OTU richness decreased from about 100 to 60.

The soil respiration and C_{min} data associate higher fungal OTU richness with faster decomposition, particularly in the black clay soil; however, other fungal and environmental variables were also significant predictors of decomposition rate. Soil respiration in the month of fungal sampling was correlated with soil type (Table 5; respiration was higher in sandy loam) and decreased with increasing soil organic C/N ($r = -0.72$). C_{min} rate increased with higher microbial biomass (including bacterial biomass), explaining 77% of C_{min} variation across both soils (see Fig. S6 in the supplemental material). C_{min} rate was also positively correlated with soil SOC, soil N, CO_2 treatment level, and Chytridiomycota relative abundance. Of all the fungal variables measured, only the relative abundance of chytrids was a significant predictor of decomposition rate.

We examined pairwise correlations among all measured fungal and environmental variables (see Appendix S1 in the supplemental material) to find other environmental predictors of fungal responses that could influence decomposition. Predictors with significant ($P < 0.05$) correlations are shown in Table 5. Basidiomycota relative abundance decreased with higher organic C/N and increased with higher C_{min}/SOC (more labile SOC). Ascomycota relative abundance is not

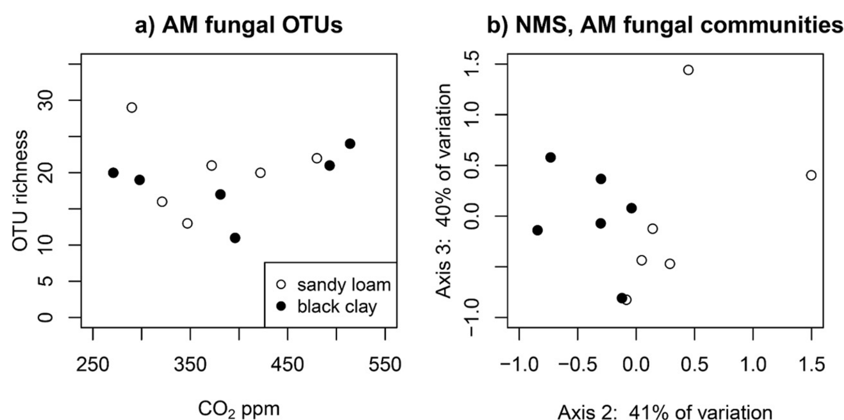

FIG 5 AM fungal OTU richness (a) and community composition (b) in sandy loam and black clay soils. OTU richness did not differ significantly by soil type or CO_2 concentration. Community composition differed marginally by soil type ($P = 0.06$). AM fungal OTUs are defined at 95% similarity.

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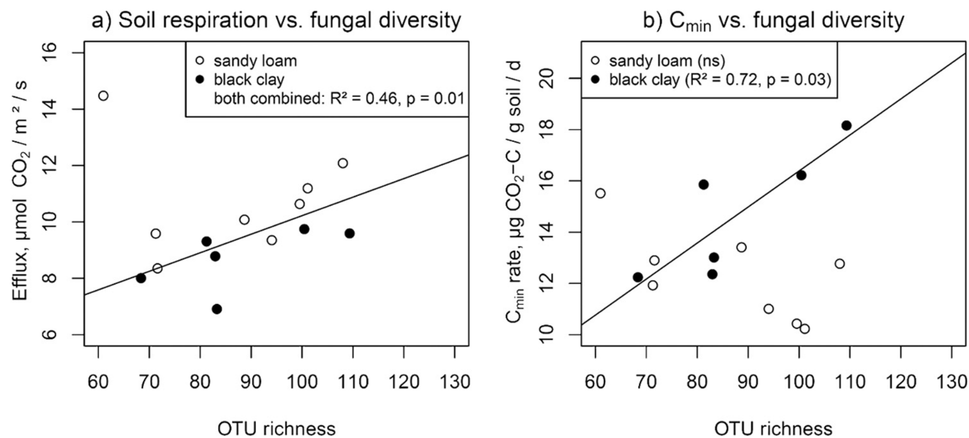

FIG 6 Fungal OTU richness as a predictor of decomposition rate, measured by July 2009 soil respiration rate (a) and potential carbon mineralization rate (b). Soil respiration data pooled from both soils fit a linear regression ($R^2 = 0.46$, $P = 0.01$). Soil respiration rate did not respond to OTU richness in the black clay or sandy loam separately, but in the sandy loam it fits a linear regression when the outlier (14.5 μmol $CO_2/m^2/s$) is removed. Potential carbon mineralization rate in the black clay (b) fits a linear regression with a positive slope. A linear regression with a negative slope is marginally significant in the sandy loam ($R^2 = 0.43$, $P = 0.08$).

correlated with any measured variable, although it is negatively correlated with Basidiomycota abundance (not shown). Chytridiomycota relative abundance was associated with higher microbial biomass and higher decomposition rate (C_{min}). Glomeromycota relative abundance was correlated with 8 factors and was one of the few fungal indicators correlated with soil type (AM fungi were less abundant in black clay). AM fungi increased with ANPP and P uptake, consistent with the hypothesis that elevated CO_2 increased plant P limitation, favoring mycorrhizae. AM fungi decreased with greater microbial biomass, greater SOC, and greater soil N. AM fungi increased with more labile SOC (C_{min}/SOC). Dominant fungal OTU9, a basidiomycete, was associated with black clay soil and increased with greater SOC and soil N.

Although the relative abundance of AM fungal species varied along the CO_2 gradient, the variation did not support our prediction that declining soil N would favor *Scutellospora* dominance at elevated CO_2. All sequences in the AM fungal data set were *Glomus*-like. The dominant AM fungal OTU was equally dominant in the two soil types (Fig. 7). AM fungal OTU4 composed between 10 to 70% of each AM fungal library. OTU4 responded differently to CO_2 in each soil, peaking at about 350 ppm in the sandy loam and about 400 ppm in the black clay. The closest BLAST match to OTU4 is an uncultured *Glomus* from soil of *Bromus rubens* (red brome) (Table 4), while a phylogenetic analysis placed OTU4 in the revised genus *Claroideoglomus* (see Fig. S3 in the supplemental material). The second and third most abundant AM fungal OTUs (OTU18 and OTU9, not shown) grouped with genus *Glomus* and genus *Funneliformis*, respectively, according to the revised taxonomy.

DISCUSSION

Can soil type inform our predictions of fungal responses to rising atmospheric CO_2? In a CO_2 gradient experiment, we found that

TABLE 5 Significant correlations[a] between fungal relative abundances, decomposition rates, and environmental factors

Environmental factor	Description of environmental factor	Fungal relative abundance[b]					Decomposition rate[c]	
		Basidio	Asco	Chytridio	Glomero	OTU9	Soil resp	C_{min}
Soil	Soil type (sandy loam = 0, black clay = 1)				−0.58	0.76	−0.55	
CO_2	CO_2, ppm							0.55
ANPP	Aboveground net primary productivity, 2009, g m^{-2}				0.56			
P uptake	Total P uptake by aboveground plant biomass, 2009, g m^{-2}				0.57			
C_{min}	C_{min} rate, mg C g^{-1} soil day^{-1}			0.57				
Mbiomass	Active microbial biomass, mg C g^{-1} soil			0.62	−0.54			0.88
SOC	Soil organic carbon, g kg^{-1}				−0.6	0.72		0.57
Total N	Total soil N, g kg^{-1}				−0.6	0.68		0.63
Organic C/N	C/N of soil organic matter	−0.53					−0.72	
Mbiomass/SOC	Index of SOC lability				0.61			
C_{min}/SOC	Index of SOC lability	0.55			0.67			
Chytridio	Chytridiomycota relative abundance							0.57

[a] Significant at P values of <0.05 ($n = 14$, $r > 0.53$ or < -0.53).
[b] Relative abundances of Basidiomycota, Ascomycota, Chytridiomycota, Glomeromycota, and relative abundance of dominant OTU9.
[c] Soil respiration rate and soil carbon mineralization rate.

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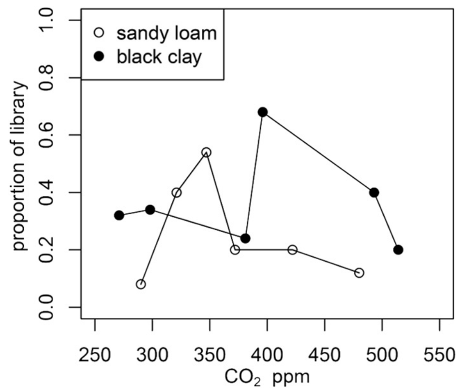

FIG 7 The relative abundance of the dominant AM fungal OTU4 as a function of CO_2 level. A phylogenetic analysis placed AM fungal OTU4 in the genus *Claroideoglomus*.

CO_2 effects on fungal communities differed significantly by soil type. Total fungal species richness as well as Chytridiomycota relative abundance increased with elevated CO_2 in the black clay soil, whereas Glomeromycota (arbuscular mycorrhizal fungi) relative abundance increased with elevated CO_2 in the sandy loam soil (Fig. 1 and 4). Likewise, fungal community composition shifted overall with CO_2 in the black clay soil (Fig. 2B). These findings support our predictions that fungal species richness would increase with elevated CO_2 in the black clay and that mycorrhizal abundance would increase with elevated CO_2 in the sandy loam. However, there was no evidence for a predicted shift in AM fungal community composition with elevated CO_2. Also, our data support the prediction that decomposition rates would increase with higher fungal species richness, but the mechanism behind this effect is unclear.

Fungal responses to CO_2 were often linear in this ecosystem, similar to microbial responses to CO_2 gradients in some (53, 54) but not all studies (55). In our study, both total fungal species richness and the relative abundance of Glomeromycota and Chytridiomycota showed linear increases with CO_2. For comparison, in a chaparral exposed to a CO_2 gradient, AM fungal abundance increased linearly from 250 to 650 ppm CO_2 but declined at 750 ppm CO_2. In our system, AM fungal relative abundance increased about 15-fold in the sandy loam soil between 290 to 480 ppm CO_2 (Fig. 4). Given the chaparral result, it is possible that CO_2 levels higher than those we examined produce threshold responses in fungi. Microbial responses to CO_2 were nonlinear in a previous CO_2 gradient experiment at the same location as our current experiment. Microbial biomass and soil respiration rates peaked at ~450 ppm CO_2 (55). We acknowledge that relative abundance responses to the CO_2 gradient have some ambiguity; a relative increase in chytrids could be due to an actual increase in chytrids and/or decrease in other taxa. However, relative abundances are commonly used to represent taxonomic shifts in 454 pyrosequencing studies (17, 37, 38, 56). The relative abundance of soil chytrids has also increased with elevated CO_2 ($P = 0.05$) in aspen stands (57).

Mechanisms of fungal community response. In our study, the increase of Glomeromycota with CO_2 in sandy loam soil supports the hypothesis that plants, experiencing greater productivity and soil P limitation at elevated CO_2, invested more C in mycorrhizae. Aboveground net primary productivity (ANPP) increased linearly with CO_2 in 2008 and 2009 in the sandy loam but not the black clay (58), and alkaline phosphatase activity (September 2009) increased with CO_2 concentration in the sandy loam, suggesting P limitation (25). Glomeromycota relative abundance increased with plant P uptake across both soils (Table 5). Experiencing CO_2-fertilized growth, plants may have allocated more carbon to mycorrhizae in the sandy loam to alleviate P limitation. In addition, the dominant, obligately mycorrhizal grass *Sorghastrum nutans* became a larger percentage of the sandy loam plant community at elevated CO_2, which suggests increased mycorrhizal abundance at elevated CO_2 (26, 31).

In contrast, the increase in total fungal species richness with CO_2 in the black clay supports the prediction that elevated CO_2 effects on the soil ecosystem were mediated by increasing availability of soil organic C. Labile C pools (including coarse particulate organic matter measured by soil physical fractionation and an active C pool measured by incubation) increased more with CO_2 in the black clay than in the sandy loam (Procter et al., unpublished). This is likely due to the higher clay content in the black clay, which protects organic matter from decomposition that would otherwise reduce the elevated CO_2 signal in soil C accumulation. Similar to labile C pools, total fungal species richness increased from about 75 to 100 OTUs across the CO_2 gradient in the black clay but did not respond significantly to CO_2 in the sandy loam (Fig. 1). SOC lability appeared to structure fungal diversity only within the black clay soil, not between the soil types. Although SOC was three times more labile in the sandy loam than the black clay, fungal OTU richness was similar in both soils and increased linearly with SOC lability in the black clay only (see Fig. S5 in the supplemental material). Relative to ambient CO_2, on average 23 new fungal OTUs may have colonized the black clay soil at elevated CO_2, possibly due to increased root growth and labile C availability presenting a larger surface to colonize. In contrast, on average 6 OTUs may have been lost from the black clay at subambient CO_2, perhaps due to greater competition for labile C.

We acknowledge several limitations to the fungal species richness patterns we observed. It is possible that fungal species were not gained or lost from the system but simply increased or decreased in abundance relative to the detection threshold of PCR-based methods. Moreover, although species richness has been used to indicate soil fungal responses to pH (17) and depth (38) gradients, species richness combines fungi of very different ecological functions. In our study, species richness includes saprotrophs, plant symbionts, and plant parasites that may respond to the CO_2 gradient through different mechanisms. Symbionts would respond to C availability within plants and how much C plants allocate to the symbiont. Saprotrophs would respond to C availability in the soil, which is affected by rhizodeposition. Saprotrophs might take longer to respond to CO_2 than plant symbionts, because the CO_2 signal would take time to enter the soil. Parasites might respond to CO_2 more like symbionts, since they are directly connected to roots. Across these diverse fungal groups, we suggest that increased labile C availability belowground leads to greater species richness, because all are dependent on an external organic carbon source. Whether belowground C availability directly or indirectly caused shifts in fungal species richness remains uncertain, as we did not independently manipulate belowground C. As is common at this sequencing level for soil fungi (36, 38, 59), our rarefaction curves did not plateau, which could create

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bias in species richness patterns. Further sequencing could therefore reveal additional diversity patterns.

The AM fungal data set did not support the hypothesis of nitrogen-mediated AM fungal community shifts along the CO_2 gradient. Although some studies have found genus-level shifts in AM fungal communities with elevated CO_2 (18, 54, 60), AM fungal communities in both soils we studied appeared to be exclusively genus *Glomus* (or *Glomus*-like genera, using the revised taxonomy). This low-diversity community is unexpected, given other studies showing that *Glomus* is associated with plants in clay-rich soil, whereas the genera *Gigaspora* and *Scutellospora* are often associated with plants in sandy soils (19–21). However, *Glomus* is often the dominant AM fungal genus in grasslands (61, 62). AM fungal relative abundance increased with lower soil N, consistent with plants investing more in mycorrhizae when N is limited (Table 5). Nitrogen was nearly three times less concentrated in the sandy loam than the black clay soil. Beyond this soil type effect, elevated CO_2 may not have induced enough N limitation to affect AM fungal community composition. Although there was evidence of N limitation in the black clay soil (25, 26), there was no significant trend of N uptake in aboveground biomass along the CO_2 gradient (see Table S3 in the supplemental material).

Because fungal species differ in pH tolerance, changes in soil pH could drive fungal community change. Soil pH declined by 0.14 units over the CO_2 gradient (see Fig. S7 in the supplemental material). In the black clay, higher fungal diversity was associated with more acidic soil. In contrast, fungal OTU richness increased with more basic soil in an artificial pH gradient (the Rothamsted Hoosfield acid strip) (17). It is possible that the small range of pH in our experiment obscures effects seen in the large range of the artificial pH gradient (pH 4 to 8.3). As with belowground C availability, we cannot prove causal linkage between soil pH and fungal community change.

Connecting fungal diversity to function. Fungal diversity has been positively associated with ecosystem functions such as productivity and decomposition rate, but mostly at low levels of diversity, such as 15 or fewer species (63–65). A microcosm study of forest soil fungi found that decomposition rate increased with fungal diversity between 1 to 12 species but that higher levels of diversity (24 and 43 species) were functionally redundant (64). Because the communities we studied contained between 60 and 110 OTUs, some of this fungal diversity may be functionally redundant. Most of the fungal OTUs are likely saprotrophic, as cellulolytic ability is widespread among Ascomycota and Basidiomycota (3), the two dominant phyla in the CO_2 gradient. Most soil chytrids are also saprotrophs, but some are plant, animal, or fungal parasites (66, 67). Many of the OTUs may be dormant propagules, as suggested in another pyrosequencing study of grassland soil fungi (38). Nevertheless, soil respiration rate in both soils, and potential C mineralization rate in the black clay, increased linearly with total fungal OTU richness (Fig. 6). These data support functional nonredundancy among fungal species. These data also support our prediction that greater soil carbon availability at elevated CO_2 allowed more fungal species to colonize the black clay and stimulated greater decomposition rate. It is possible that increased fungal OTU richness at elevated CO_2 caused more efficient decomposition due to fungal species having complementary enzymes. Alternatively, CO_2 may have increased decomposer taxa; chytrid relative abundance increased with CO_2 and was correlated with a higher C_{min} rate (Fig. 4 and Table 5). However, the effect of fungal diversity on decomposition could be confounded with the effect of total microbial biomass, which also increased with elevated CO_2 in the black clay but not the sandy loam (Procter et al., unpublished). Microbial biomass explained 77% of the variation in potential C mineralization rate across both soils, while fungal OTU richness explained 72% of the variation in potential C_{min} in the black clay only (Fig. 6; see also Fig. S6 in the supplemental material). Therefore, the species in our fungal communities may exhibit some nonredundancy in decomposition function, but it is difficult to distinguish the effect of fungal diversity from that of microbial biomass on decomposition rate. A study on bacterial diversity responses to the CO_2 gradient would provide insights into this question.

There is likely both functional redundancy and functional difference among the AM fungal taxa in the CO_2 gradient. All were *Glomus* or *Glomus*-like, a genus recently reclassified into two families containing five genera (45). Species under the old definition of *Glomus* occupy a "low-carbon" niche, having most of their hyphae within roots, an adaptation for extracting root carbon. Their cells contain vesicles for carbon storage in times of carbon limitation (18). That said, *Glomus* species can differ in root colonization, (68), effects on plant growth (63), and affinity to host plant species (39). Among the AM fungal genera in the CO_2 gradient, the largest phylogenetic division is between *Claroideoglomus* and the other three genera (see Fig. S3 in the supplemental material), as *Claroideoglomus* is within a separate family (45). The dominant AM fungal OTU4 is within *Claroideoglomus*. Therefore, there may be functional differences between OTU4 and other AM fungal genera in the CO_2 gradient. For instance, Gamper et al. (68) found that a *Claroideoglomus* sp. colonized less root length than a *Rhizophagus* sp. (formerly *Glomus intraradices*). OTU4 could be less demanding of root C than other AM fungi in the CO_2 gradient and could have a competitive advantage at lower CO_2 levels, where root C is less plentiful.

Soil type effects on community composition. It is difficult to identify the mechanism by which soil type affects fungal community response to CO_2, since multiple, often correlated factors could be involved (pH, SOC, texture, moisture, nutrient availability) (16). In a study of elevated CO_2 experiments in five ecosystems, Weber et al. suggest that the unique soil properties of a marsh explained its unique soil fungal community (3). The marsh soil had a distinct fungal cellulolytic gene community compared to those of the other ecosystems, as well as the highest moisture, SOC, and nutrient availability (3). However, without a manipulation experiment, it is difficult to determine which soil properties most influenced the fungal community. The two soils in our experiment had similar fungal species richness to one another but differed in community composition and response to CO_2 (Fig. 1, 2, and 4). In our study, the higher-clay soil had significantly higher organic C, total N, and organic C/N. Basidiomycota relative abundance increased with lower organic C/N, while Glomeromycota relative abundance increased with lower organic C and total N (Tables 2 and 5). Higher-clay soils tend to accumulate organic matter, due to low aeration and physical protection of organic matter, which inhibit decomposition compared to that of low-clay soils (69, 70). Because the two soils in our experiment differed little in pH and N availability (26), it is likely that fungal community differences by soil type are due to organic matter type and availability, as regulated by soil texture. Soil moisture may also have played a role; due to increased plant water use efficiency, soil water potential increased more with elevated CO_2 in the black clay than the sandy loam

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(26). This result may explain the increase in fungal diversity with CO_2 in the black clay, as higher moisture could have stimulated plant productivity and/or decomposability of soil organic matter, allowing more fungal species to grow.

Elevated CO_2 studies that involve more than one soil type are rare but show soil type effects on plant productivity, fungal hyphal length, and microbial decomposition rates (22, 23). In annual grasslands, AM fungal hyphal length increased with elevated CO_2 in a nutrient-rich soil but not a nutrient-poor soil (22). AM fungal abundance at our site also increased with elevated CO_2, but in the lower-nutrient sandy loam rather than the black clay (Fig. 4). In forest mesocosms, soil microbial decomposition rate varied significantly by soil type but not by CO_2 treatment (23). In contrast, our study suggested that CO_2 treatment increased decomposition rate, but only in the black clay soil. Although soil type is rarely discussed in the elevated CO_2 literature, field-scale elevated-CO_2 experiments represent a texture range of at least 6 to 55% clay and 10 soil orders (Procter et al., unpublished). Our study suggests that soil type could help explain why fungal responses to CO_2 vary, but further experiments are necessary to understand the mechanisms involved.

Conclusion. We found that soil type significantly structured fungal communities and, in several cases, their responses to elevated CO_2. The CO_2 gradient experiment in this grassland ecosystem demonstrated that total fungal species richness and the relative abundance of two fungal phyla (Glomeromycota, Chytridiomycota) increased linearly with CO_2 concentration in the 250 to 500 ppm range. However, the sensitivity of these groups to CO_2 depended on soil type, as did the response of overall fungal diversity. Connecting diversity to function is challenging, but fungal species richness was positively associated with decomposition rate, particularly in the clay-rich soil. We suggest soil type affects fungal community composition and its response to CO_2 through texture-mediated differences such as organic matter availability and quality. Because soil type varies considerably across landscapes, soil type could be an important predictor of fungal responses to CO_2.

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