Soil Microbial Community Responses to Multiple Experimental Climate Change Drivers\(^\dagger\)\(^\ddagger\)

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Researchers agree that climate change factors such as rising atmospheric \([CO_2]\) and warming will likely interact to modify ecosystem properties and processes. However, the response of the microbial communities that regulate ecosystem processes is less predictable. We measured the direct and interactive effects of climatic change on soil fungal and bacterial communities (abundance and composition) in a multifactor climate change experiment that exposed a constructed old-field ecosystem to different atmospheric \(CO_2\) concentration (ambient, +300 ppm), temperature (ambient, +3°C), and precipitation (wet and dry) might interact to alter soil microbial and fungal abundance and community structure in an old-field ecosystem. We found that (i) fungal abundance increased in warmed treatments; (ii) bacterial abundance increased in warmed plots with elevated atmospheric \([CO_2]\) but decreased in warmed plots under ambient atmospheric \([CO_2]\); (iii) the phylogenetic distribution of bacterial and fungal clones and their relative abundance varied among treatments, as indicated by changes in 16S rRNA and 28S rRNA genes; (iv) changes in precipitation altered the relative abundance of Proteobacteria and Acidobacteria, where Acidobacteria decreased with a concomitant increase in the Proteobacteria in wet relative to dry treatments; and (v) changes in precipitation altered fungal community composition, primarily through lineage specific changes within a recently discovered group known as soil clone group I. Taken together, our results indicate that climate change drivers and their interactions may cause changes in bacterial and fungal overall abundance; however, changes in precipitation tended to have a much greater effect on the community composition. These results illustrate the potential for complex community changes in terrestrial ecosystems under climate change scenarios that alter multiple factors simultaneously.

Soil microbial communities are responsible for the cycling of carbon (C) and nutrients in ecosystems, and their activities are regulated by biotic and abiotic factors such as the quantity and quality of litter inputs, temperature, and moisture. Atmospheric and climatic changes will impact both abiotic and biotic drivers in ecosystems and the response of ecosystems to these changes. Feedbacks from ecosystem to the atmosphere may also be regulated by soil microbial communities (3). Although microbial communities regulate important ecosystem processes, it is often unclear how the abundance and composition of microbial communities correlate with climatic perturbations and interact to effect ecosystem processes. As such, much of the ecosystem climate change research conducted to date has focused on macroscale responses to climatic change such as changes in plant growth (43, 44), plant community composition (2, 37), and coarse scale soil processes (14, 18, 21, 26), many of which may also indirectly interact to effect microbial processes. Studies that have addressed the role of microbial communities and processes have most often targeted gross parameters, such as microbial biomass, enzymatic activity, or basic microbial community profiles in response to single climate change factors (22, 28, 29, 33, 61, 63).

Climate change factors such as atmospheric \(CO_2\) concentrations, warming, and altered precipitation regimes can potentially have both direct and indirect impacts on soil microbial communities. However, the direction and magnitude of these responses is uncertain. For example, the response of soil microbial communities to changes in atmospheric \(CO_2\) concentrations can be positive or negative, and consistent overall trends between sites and studies have not been observed (1, 28, 34–36). Further, depending on what limits ecosystem productivity, precipitation and soil moisture changes may increase or decrease the ratio of bacteria and fungi, as well as shift their community composition (8, 50, 58). Increasing temperatures can increase in microbial activity, processing, and turnover, causing the microbial community to shift in favor of representatives adapted to higher temperatures and faster growth rates (7, 46, 60, 64, 65). Atmospheric and climatic changes are happening in concert with one another so that ecosystems are experiencing higher levels of atmospheric \(CO_2\), warming, and changes in precipitation regimes simultaneously. Although the many single factor climate change studies described above have enabled a better understanding of how microbial communities may respond to any one factor, understanding how multiple climate change factors interact with each other to influence microbial community responses is poorly understood. For example, elevated atmospheric \([CO_2]\) and precipitation changes might increase soil moisture in an ecosystem, but this increase may be counteracted by warming (10). Similarly, warming may increase microbial activity in an ecosystem, but this increase may be eliminated if changes in precipitation...
lead to a drier soil condition or reduced litter quantity, quality, and turnover. Such interactive effects of climate factors in a multifactorial context have been less commonly studied even in plant communities (45), and detailed studies are rarer still in soil microbial communities (25). Clearly, understanding how microbial communities will respond to these atmospheric and climate change drivers is important to make accurate predications of how ecosystems may respond to future climate scenarios.

To address how multiple climate change drivers will interact to shape soil microbial communities, we took advantage of a multifactor climatic change experiment that manipulated atmospheric CO₂ (+300 ppm, ambient), warming (+3°C, ambient) and precipitation (wet and dry) in a constructed old-field ecosystem that had been ongoing for 3.5 years at the time of sampling. Previous work on this project has demonstrated direct and interactive effects of the treatments on plant community composition and biomass (15, 30), soil respiration (56), microbial activity (30), nitrogen fixation (21), and soil carbon stocks (20). These results led us to investigations of how the soil bacterial and fungal communities, important regulators of some of these processes, were responding using culture-independent molecular approaches. Our research addresses two overarching questions. (i) Do climatic change factors and their interactions alter bacterial and fungal abundance and diversity? (ii) Do climatic change factors and their interactions alter bacterial or fungal community composition?

MATERIALS AND METHODS

Site description and experimental design. The Old-Field Community Climate and Atmospheric Manipulation (OCCAM) was established in 2002 on the National Ecological Research Park, Oak Ridge, TN (35°54'N, 84°20'W). The site was in agricultural use until 1943 (10, 15, 20, 21, 30, 45). The soils are of alluvial origin and classified as Captina silt loam (12). The mean January minimum temperature is −2.7°C, and the mean July maximum temperature is 31.2°C, with an annual mean precipitation of 1,322 mm, uniformly distributed throughout the year (56). The experimental setup was described previously in Wan et al. (56) and Garten et al. (21). Briefly, three blocks with five plots each were trenched to 75 cm and lined with polyvinyl chloride film and foam insulation to create 15 m²-diameter plots. Existing vegetation and most of the seed bank was removed. All sequences used in the analysis were partial sequences of a length of 400 to 500 bp. Bacterial and acidobacterial clones were taxonomically assigned by using NCBI BLAST database (http://www.ncbi.nlm.nih.gov/BLAST). Bacterial 16S rRNA genes were amplified by using the universal 16S primers 8F and 1492R (32). The reaction mixture included 2 μl of 10× PCR buffer, 1.6 μl of 25 mM MgCl₂, 2 μl of a 10-ng ml⁻¹ BSA mixture, 1.6 μl of 25 mM (dATP, dCTP, dGTP, dTTP) and 1 μl of a 20-pmol μl⁻¹ mixture of each primer, 1 μl of 1:10-diluted soil DNA solution, 0.3 μl of Taq polymerase, and H₂O to a final volume of 20 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, Norwalk, CT). Acidobacterial rRNA genes were amplified by using ACD31F (5) and 1492R using identical reaction conditions to the bacterial PCR amplifications. Fungal 18S rRNA gene amplifications were performed using ITS9F (13) and LSU1221R (49) primers that amplify a region including the ITS1, 5.8S rRNA, ITS2, and the 5' end of the 28S rRNA gene. The reaction mixture contained 2 μl of 10× PCR buffer, 2.2 μl of 2.75 mM MgCl₂, 2.5 μl of a 10-ng ml⁻¹ concentration of BSA, 1 μl of 10-pmol μl⁻¹ concentration of each primer, 1.6 μl of 25 mM dNTPs, 0.6 μl of Taq polymerase, and 1 μl of 1:10-diluted soil DNA solution and H₂O to a final volume of 20 μl. The reaction was carried out under the following conditions: 94°C for 2 min; followed by 25 cycles of 94°C for 45 s, 57.5°C for 50 s, and 72°C for 1 min 45 s (+1 s each cycle); and finally 72°C for 5 min. Positive and negative controls were used in all PCRs. Electrophoresis was performed in 1% agarose in Tris-acetate EDTA buffer to confirm successful amplification, and PCR products were extracted from the agarose gel by using a gel purification kit (Qiagen, Valencia, CA). PCR products from the wet and dry sides of the three blocks for each plot were performed using TA primers (48) for bacterial and acidobacterial libraries and LROF (55) for fungal libraries using the following master mix: 1 μl of 5× sequencing buffer, 1.7 μl of H₂O, 1 μl of BigDye, 0.3 μl of 20-pmol μl⁻¹ mixture of TA primer, and 1 μl of DNA template. Products were precipitated in ethanol and resuspended in 10 μl of Hi-Di Formamide (Applied Biosystems) and run on an ABI Prism 3730 DNA analyzer (Applied Biosystems). Sequences were edited in Sequencher program version 4.7 (Gene Codes Corp., Ann Arbor, MI), where the extents of the vector and low-quality ends were trimmed, and sequences were submitted to GenBank. All sequences included in the analysis were partial sequences of a length of 400 to 500 bp. Bacterial and acidobacterial clones were taxonomically assigned by using the Ribosomal Database Project II website (http://rdp.cme.msu.edu/) (40). Fungal clone analyses were performed by using NCBI BLAST database (http://
Acidobacterial sequences and 1% for fungal sequences. Taxonomic units (OTUs) were defined at a distance level of 3% for bacterial and 5% for fungal libraries. Bootstrap analysis was performed with 100 replications. Edited sequences were submitted to GenBank (accession numbers GU374349 to GU376452). Rooted phylogenetic tree created with BioEdit were analyzed using the Unifrac interface which provides a suite of tools to compare microbial communities. Unifrac significance tests the differences among treatments in terms of the branch which is unique to each treatment. A P-test uses the phylogenetic tree to test whether two environments are significantly different using parsimony (41). Unifrac metrics were used in jackknife environment clustering which performs statistical resampling to test the confidence of clusters, and principal coordinate analyses (PCoA) were used to find clusters and the most important axes of variation among samples. Unifrac tests were performed using 1,000 permutations and calculated with the Unifrac web application (http://bmf2.colorado.edu/unifrac) (39). Unifrac and P-test significance values were corrected by using the Bonferroni correction for multiple comparisons. Diversity indexes and rarefaction curves were calculated by using the DOTUR program (51), and operational taxonomic units (OTUs) were defined at a distance level of 3% for bacterial and acidobacterial sequences and 1% for fungal sequences.

RESULTS

Treatment effects on bacterial and fungal abundance and community phylogenetic composition metrics. Fungal abundance increase with elevated temperature, as measured by total copies of rRNA genes using quantitative PCR (P ≤ 0.09). Bacterial abundance decreased under ambient CO2, and elevated temperature decreased abundance. However, under elevated CO2 and elevated temperature, an increased bacterial abundance was observed (CO2 × temperature; P < 0.06) (see Fig. SAI in the supplemental material and Table S1 in the supplemental material). The relative fungus/bacterium ratio was not significantly different among treatments, as measured by gene copy numbers, but they were within the range previously reported for similar soils (11, 17).

For the bacterial libraries, a total of 665 rRNA gene sequences were used in the analysis (349 clones for wet plots and 316 clones for dry plots) representing one clone library per treatment (about 70 to 90 clones per library). For the fungal libraries, a total of 291 clones were used in the analysis for the wet treatments and 310 clones for the dry treatments. In the case of acidobacterial 16S rRNA sequences, 195 clones for the wet plots and 160 clones for the dry plots were used in the analysis (see Table S2 in the supplemental material). Rarefaction curves for fungal libraries from wet plots reached saturation, and libraries from dry plots started to approach saturation (data not shown). For acidobacterial libraries a similar pattern was observed but was less marked than with the fungal libraries. Not surprisingly, bacterial rarefaction curves did not reach saturation, and a more exhaustive screening of cloning would be needed. Although the recovered phylotypes reflect the major trends in the libraries, a more extensive clone screening would have been ideal. Although full sampling coverage of the diversity within the bacterial library samples was not achieved with the number of clones sequenced, clone distribution analysis performed in four individual 16S bacterial rRNA libraries from aCO2aTemp samples (technical replicates) revealed that the phylogenetic distribution and diversity metrics were consistent and reproducible between replicate libraries (see Table S2 in the supplemental material). In addition, because we were not able to saturate sampling, we restrict our discussion here to trends in high-level phylogenetic groups, rather than individual OTUs, which are likely to be undersampled.

We detected consistent and significant changes among our treatments in the 16S bacterial and acidobacterial rRNA gene, and 28S fungal rRNA gene libraries composition using Unifrac tests, P-tests, Jackknife environment clusters, and PoCA. Using Unifrac metrics, bacterial communities were significantly different under wet relative to dry treatments (Unifrac and P-test significance, P ≤ 0.028). Similar to the bacterial response, the fungal communities under dry and wet treatments also differed significantly (P ≤ 0.028).

The precipitation treatments (dry and wet) resulted in dis-
tinct clusters of bacterial, acidobacterial, and fungal clone libraries using Unifrac cluster analysis (Fig. 1). These results support our conclusions based on P-test and Unifrac analyses. Bacterial and acidobacterial sequences exhibited the highest level of Jackknife support for the distinct precipitation (dry and wet) clustering (99.95). Unifrac PoCA analysis also supported these results, where PoCA axis 1 showed 39, 30, and 33% of the variation and PoCA axis 2 showed 14, 17, and 21% of the variation in bacterial, acidobacterial, and fungal communities, respectively (Fig. 1). These results also are supported by the observed changes in representative abundance in the major clades within the fungal data set discussed below. Overall, diversity metrics did not differ within or among treatments according to Chao1, Shannon, and Simpson metrics calculated by using the DOTUR program for bacterial or acidobacterial libraries, but did show changes within fungal libraries and indicated the wet soils were much less diverse than dry soils (see Table S1 in the supplemental material). A similar clustering of communities corresponding to precipitation treatment was observed for the fungal and acidobacterial community (Fig. 1, only patterns for bacterial community are presented for clarity since fungal and acidobacterial exhibited similar patterns).

Climate change factor effects on specific bacterial or fungal phylogenetic groups. The bacterial 16S rRNA clone libraries revealed a great diversity of organisms present and a phylum-level distribution similar to previously reported soil 16S rRNA clone libraries (27). We taxonomically grouped the clone libraries of the PCR-amplified 16S bacterial rRNA genes according to RDP (Fig. 2). The majority of the sequences were related to the Proteobacteria phylum, followed by sequences related to Firmicutes, Bacteroidetes, Actinobacteria, Acidobacteria, and Planctomycetes. Minor components of the library were sequences related to the Verrucomicrobia, Chloroflexi, and Nitrospirae phyla. The relative abundance of certain groups within the bacterial community responded strongly to the precipitation treatment. Although caution is needed since the libraries are unreplicated, it seems the acidobacteria were more abundant under dry conditions—making up 26% of the community in dry plots relative to only 10% of the community in wet plots, whereas proteobacteria were more abundant under wet conditions —making up 43% of the community in dry plots relative to 61% of the community in wet plots.

The Proteobacteria phylum dominated the bacterial community across all treatments. Within the Proteobacteria, the alpha-proteobacteria were dominated by members of the Rhizobiales, the betaproteobacteria were dominated by Burkholderiales, the deltaproteobacteria were dominated by Myxococcales, Syntrophobacteriales, and Desulfobacteriales, and the gammaproteobacteria were dominated by Chromatiales and Enterobac-
bacteria. Within the *Proteobacteria* phyla, the subdivision deltaproteobacteria was present in high abundance in most of the treatments (see Fig. SA2 in the supplemental material), and it seems that the deltaproteobacteria increased under eCO₂ treatment. In the overall bacterial libraries, the smaller proportion of clones recovered from phyllogenetic groups other than *Proteobacteria* made their responses more difficult to interpret and more likely to be biased by the low sample numbers of cloned representatives.

Since overall acidobacteria were responsive to precipitation regimen and they are an understudied soil microbial group, we constructed additional clone libraries using the acidobacterial primers published by Barns et al. (4) and analyzed for changes in the relative abundance of the defined phyllogenetic subgroups also presented in that study. The relative composition of different acidobacterial lineages defined by Barns et al. (4) varied with precipitation, but not other treatments (see Fig. SA3 in the supplemental material). In all treatments, however, the dominant acidobacterial clones were related to group Gp6, followed by Gp4, Gp5, and Gp1, a finding similar to previously reported soil distributions (5), including samples reported previously from subsurface soils near Oak Ridge (4). Gp6 tended to increase under elevated CO₂ in wet plots and decrease under elevated CO₂ in dry plots. Other group-specific acidobacteria responses were not detected or apparent.

Specific fungal lineages also differed among the climate treatments (Fig. 3). In the wet plot libraries, the sequences were mostly related to uncultured fungal sequences in the fungal branch known as soil clone group I (SCGI) (47). These sequences represented almost 95% of the total recovered sequences in the wet treatments and exhibited some grouping based on the other climate drivers (CO₂ or temperature). Although most of the SCGI subgroups were uniformly distributed among treatments, sequences related to uncultured SCGI clone CROX-D11 (EU179550) were surprisingly dominant in the aTemp treatments. In this clade, two clear subclades were present: clade A was dominant under aCO₂ treatments, and clade B was dominant under eCO₂ treatments. Within the dry treatments, sequences related to SCGI represented 73% of the clone libraries but showed no detectable differences in the relative distribution of clones among the temperature and CO₂ treatments.

**DISCUSSION**

Atmospheric and climate change drivers and their interactions may select for distinct soil microbial communities, and these community changes may shape the way ecosystems function in the future. We took advantage of an ongoing, multifactor climate change experiment to investigate how multiple climatic change factors and their interactions impact both the abundance and the diversity of soil bacterial and fungal communities. Using molecular techniques, we targeted changes in the abundance and community composition of the bacterial, acidobacterial, and fungal components of the soil microbial community. Our treatments did not impact fungal to bacteria relative ratio, but bacterial abundance was affected by an interactive effect of CO₂ and temperature, and fungal abundance was affected by temperature but not precipitation or CO₂ regime. In addition, there were changes in the relative composition of bacterial and fungal groups, and these shifts were largely shaped by the precipitation regime.

**Do climatic change factors interact to affect bacterial and fungal abundance and community diversity metrics?** Fungal abundance was responsive to elevated temperature, and bacterial abundance was responsive to interactive effect of CO₂ and temperature. However, there were no detectable direct or interactive impacts on the fungus/bacterium ratio among our climate change treatments. Our results are in agreement with those of previous reports. For example, researchers have found that changes in CO₂ do not predictably alter microbial biomass (35, 62). Similarly, changes in soil moisture and ecosystem warming do not always lead to predictable or significant changes in bacterial and fungal abundance (8, 19, 29, 58). Previous reports in this site have shown a response of some plants to a warming effect, in particular *Solidago* and *Andropogon*, with some other plant productivity being reduced; however, the response is complicated by time of sampling (15). Therefore, our quantitative PCR data may be revealing a fungal communities response to temperature that can be the result of direct effects of temperature in the fungal community and/or an indirect effect by responding to plant community changes. The bacterial response to interactive effects of CO₂ and temperature may represent a more complex scenario. It is possible that a lack of labile substrates under elevated temperature alone favors fungal over bacterial community members, while increased substrate availability from plants under elevated CO₂ allows increases in bacterial abundance with increased temperature.

Major changes in the bacterial and fungal libraries were observed in the distribution of phylogenetic affiliation of clones, and these changes were mostly influenced by the precipitation treatment rather than CO₂ or temperature treatments. The precipitation treatment also had the largest influence relative to temperature and atmospheric [CO₂] on plant community biomass and plant community composition in these experimental ecosystems (30). Precipitation-driven shifts in plant community composition may thus alter the amount and quality of litter inputs into the soil ecosystem, and these changes may indirectly alter the soil microbial community. Our precipitation treatment had the largest impact on microbial community composition, and this result was confirmed by using several phylogeny-based comparison tools in UniFrac (38). Soil moisture can impact the physiological status of soil microorganisms, physicochemical properties of soils, and plant productivity. Increased plant productivity may result in greater inputs of plant-derived carbon in soils, which may alter soil microbial communities. The quality and quantity of the plant-derived carbon substrates (litter, dead roots, and roots exudates) may be affected by moisture resulting in different microbial communities. Alternatively, microbial communities could be responding directly to soil moisture, which was quite different between the wet and dry treatments (10). The response to elevated CO₂ or temperature was relatively small and insignificant for community composition metrics; however, minor responses were observed for particular groups such as deltaproteobacteria or fungal sequences related to the uncultured SCGI clone CROX-D11 discussed below, indicating that perhaps temperature and CO₂ do not play the major role in affecting microbial composition, which is in agreement with
previous studies (8, 19, 29, 36, 58, 60). Unfortunately, due to a lack of available resources, we were not able to take full advantage of the replication of this multifactor experiment and were forced to pool replicates across the treatments in order to sample the microbial community broadly (Bacteria, Fungi, and Acidobacteria) so our ability to use more traditional statistical methods across treatments is somewhat limited beyond the phylogenetic-distance-based methods described above.

FIG. 3. Fungal rRNA neighbor-joining tree of SCGI clones and distribution of different subclades recovered from each treatment for wet plots. Scale bars represent 2% changes. Numbers at the nodes represent bootstrap resampling support based on 100 replicates; only values >50 are presented.
Do climatic change factors differentially affect bacterial or fungal phylogenetic groups? The relative abundance of bacterial phyla was clearly impacted by the precipitation treatment, which led to shifts in the relative abundance of Proteobacteria and Acidobacteria. The other two factors, CO₂ and temperature, did not have a major impact on the distribution of these groups. The relative abundance of Proteobacteria was greater in the wet relative to the dry treatments, whereas Acidobacteria abundance was greater in dry treatments.

Surprisingly, within the Proteobacteria phylum, the predominant class was the deltaproteobacteria, a surprising result since most of soil proteobacteria are dominated by the α, β, and γ classes. Deltaproteobacteria recovered from these soils consisted mainly of members of the Myxococcales and Syntrophobacterales orders. Myxococcales are typically found in soils and exhibit an aerobic organotrophic metabolism with some representatives able to utilize cellulose (9); however, their dominance is unexpected. The presence of Syntrophobacterales in these aerobic soils is also surprising, since their metabolism is usually anaerobic (31). The dominance of the deltaproteobacterial class could be related to a specific microorganism-plant interaction in this old field design, but the reasons still remains unclear. Acidobacteria, a very ubiquitous phylum in soils, were most predominant within the dry treatment soils. Despite their ubiquity in soils, very little is known about their metabolism, and very few members of this group are cultured or studied (5). Recently, 26 subdivisions for the Acidobacteria phyla were proposed (4), and three genomes from cultured representatives have been sequenced (57). According to the genome data, these organisms possess the ability to utilize a broad range of substrates and exhibit genetic traits typical of microorganisms able to survive desiccation stress and withstand fluctuations in soil moisture(57). Moreover, acidobacteria tend to prefer oligotrophic niches (16), suggesting that they may be favored in dry soils, where a lower plant metabolism and productivity may result in lower availability of plant-derived carbon sources, possibly decreased dissolved organic carbon levels, and generally more oligotrophic conditions. Fierer et al. (16) reported that members of the Acidobacteria tend to be favored in oligotrophic soils with lower carbon availability, and betaproteobacteria were favored in copiotrophic conditions, but these authors did not study the behavior of delta-proteobacteria. Moreover, it has been proposed that the ratio of acidobacteria to proteobacteria could be used as indicator of the trophic level of soils (52), and the ratios we observed would thus suggest that the dry treatments are indeed more oligotrophic than the wet treatments. Garten et al. (20) showed that in these sites reduced soil moisture slowed carbon turnover in the particulate organic matter pool, which can reduce substrate availability and result in more oligotrophic conditions. In another multifactorial global change experiment at Jasper Ridge in central California, ammonia-oxidizing bacteria, betaproteobacteria responsible for the oxidation of ammonia in nitrification, were shown to decrease in response to elevated CO₂ and the decrease was more pronounced with elevated precipitation (25). In our study, deltaproteobacteria also seemed to respond positively to elevated CO₂ in the dry and wet treatments, similarly to previous studies in chaparral ecosystems (36).

Although the total number of acidobacterial clones in the overall 16S bacterial libraries differed among wet and dry treatments, the distribution of the clones within separate specific acidobacterial libraries was not affected by climate treatments, which may indicate shifts in overall abundance but not particular selection for any subdivision of Acidobacteria among the different climate treatments. Clearly, acidobacteria are affected by soil moisture and, given their high abundance in many environments worldwide, should be further studied with methods specific to this group to better understand their participation and roles in soil processes.

Within the fungal libraries, most of the sequences were associated to the uncultured fungal SCGI that is known only from rRNA gene sequence data but represents a major sub-phylum level lineage within the Ascomycota (47). SCGI sequences were more abundant in wet treatments (95%) than in dry treatments (73%) in the present study. These relative abundances are much higher than the range of previous reported values (7 to 27%) (47, 54) despite the use of PCR and other laboratory methods identical to our previous studies. We are unsure how exactly to interpret this extremely high abundance given the unknown nature of the physiology and ecology of these organisms; however, such high levels of SCGI suggest a specific relationship of SCGI taxa with these soil types or perhaps a specific interaction with one or more of the plants present in our experiment. This group has always previously been found to be associated with active plant rhizosphere (Porter et al. [47]), and since rhizosphere dynamics such as carbon exudation, root proliferation, and turnover, and other belowground processes may be impacted by climatic change, this fungal group could be an important indicator species and should be targeted in future research.

Conclusions. Our results demonstrate that the direct and interactive impacts of climatic change will likely reshape bacterial and fungal soil communities and that changes in precipitation in particular will be important in dictating the response of microbial community composition in the future. Further, we found that while fungal abundance overall responds to temperature and bacterial abundance to interactive effects of CO₂ and temperature and not to direct or indirect effects of precipitation regime, changes in bacterial and fungal community composition were readily apparent with precipitation regime. Our data highlight the need to know more about the physiology and ecology of the various uncultured, dominant, and responsive soil community members, such as SCGI and acidobacteria, and how these organisms will potentially be affected by, and feedback to, the response of ecosystems to climatic change.

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