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Drought consistently alters the composition of soil fungal and bacterial communities in grasslands from two continents

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Abstract

The effects of short-term drought on soil microbial communities remain largely unexplored, particularly at large scales and under field conditions. We used seven experimental sites from two continents (North America and Australia) to evaluate the impacts of imposed extreme drought on the abundance, community composition, richness, and function of soil bacterial and fungal communities. The sites encompassed different grassland ecosystems spanning a wide range of climatic and soil properties. Drought significantly altered the community composition of soil bacteria and, to a lesser extent, fungi in grasslands from two continents. The magnitude of the fungal community change was directly proportional to the precipitation gradient. This greater fungal sensitivity to drought at more mesic sites contrasts with the generally observed pattern of greater drought sensitivity of plant communities in more arid grasslands, suggesting that plant and microbial communities may respond differently along precipitation gradients. Actinobateria, and Chloroflexi, bacterial phyla typically dominant in dry environments, increased their relative abundance in response to drought, whereas Glomeromycetes, a fungal class regarded as widely symbiotic, decreased in relative abundance. The response of Chlamydiae and Tenericutes, two phyla of mostly pathogenic species, decreased and increased along the precipitation gradient, respectively. Soil enzyme activity consistently increased under drought, a response that was attributed to drought-induced changes in microbial community structure rather than to changes in abundance and diversity. Our results provide evidence that drought has a widespread effect on the assembly of microbial communities, one of the major drivers of soil function in terrestrial ecosystems. Such responses may have important implications for the provision of key ecosystem services, including nutrient cycling, and may result in the weakening of plant–microbial interactions and a greater incidence of certain soil-borne diseases.

KEYWORDS

drought, enzyme activities, microbial community, rainfall manipulation, soil-borne pathogens

1 | INTRODUCTION

Microorganisms play fundamental roles as primary producers and decomposers and provide important ecosystem services such as organic matter decomposition and nutrient cycling and storage (Bardgett & van der Putten, 2014; van der Heijden, Bardgett, & van Straalen, 2008). The structure and activity of microbial communities in soils is influenced by substrate properties, particularly soil pH and organic matter content, and vegetation type (Delgado-Baquerizo et al., 2016a; Fierer & Jackson, 2006); at larger spatial and temporal scales long-term climatic phenomena such as increasing aridity have also been associated with lower bacterial and fungal abundance and lower microbial diversity (Maestre et al., 2015). However, much less is known about how short-term climatic processes, including the more extreme drought events forecast (Cook, Ault, & Smerdon, 2015), will affect microbial communities and the ecosystem services they mediate.

Climate models forecast widespread changes in precipitation regimes, including longer, more intense droughts (McLaughlin, 2014), causing desertification and promoting the expansion of drylands globally (Huang, Yu, Guan, Wang, & Guo, 2016). Field-based climate change studies have traditionally focused on aboveground responses (e.g., plant productivity, biomass, and community composition) of local studies, whereas belowground responses, particularly those of microbial communities, have received much less attention (Wilcox, von Fischer, Muscha, Petersen, & Knapp, 2015; Wilcox et al., 2017). Experimental approaches across multiple sites and continents, where contrasting climatic and edaphic conditions may mediate the impacts of changing precipitation regimes, are equally needed to accurately predict the response of microbial communities to extreme drought (Fridley, Grime, Askew, Moser, & Stevens, 2011; Grime et al., 2008). Drought experiments and meta-analyses consistently predict negative impacts of drought on the diversity and abundance of soil microbial communities (Wu, Dijkstra, Koch, Peñuelas, & Hungate, 2011), with bacteria typically considered more sensitive than fungi (Evans & Wallenstein, 2012; Fry et al., 2016). Given the strong link between microbial communities and soil functioning, any alteration in the composition of microbial communities due to climate change might disrupt the functioning of soil, and thus the supply of ecosystem services (Bellard, Bertelsmeier, Leadley, Thuiller, & Courchamp, 2012; McLaughlin, 2014). Because of this, improving our understanding of the role of altered precipitation regimes in the regulation of soil microbial communities is of paramount importance to accurately predict changes in terrestrial ecosystem processes linked to future climate change scenarios (Delgado-Baquerizo et al., 2016b; Maestre et al., 2015).

Grasslands are critically important components of terrestrial ecosystem feedbacks to climate change. They represent ca. 40% of the total land surface, store ca. 3.4 t C ha^{-1} $year^{-1}$ and provide multiple ecosystem services (McLaughlin, 2014). Grasslands also greatly contribute to regulate the interannual variability in the soil C sink at the global scale (Ahlström et al., 2015; Poulter et al., 2014) and, therefore, understanding how microbial community structure and functioning in grasslands respond to drought is essential for predicting impacts of climate change on the global C cycle. Climate models for the Central and Southwest US, where grasslands dominate the landscape, predict an intensification of the hydrological cycle, with high interannual precipitation variability and fewer but larger rain events (Cook et al., 2015). Similarly, for south-eastern Australia, where land conversion has transformed more than 90% of native woodlands into seminatural grasslands, the most recent climate models predict an increase in the occurrence of extreme precipitation events interspersed with longer droughts and shifts in precipitation seasonality but little change in total precipitation (McLaughlin, 2014).

In this study, we evaluated the impacts of comparable extreme drought simulation experiments (50% precipitation reduction) conducted at seven grasslands located in two continents, North America and Australia (Table 1; Figure 1). We sampled each site during the 2nd or 3rd year of imposed drought and measured (i) soil microbial community richness (number of phylotypes) and composition (relative abundance of phylotypes), (ii) microbial abundance, and (iii) the potential activities of enzymes associated with decomposition and nutrient cycling by soil microbial communities. The consistency of treatment types between sites in the USA (66% precipitation reduction during the growing season, equivalent to an annual reduction of 50%) and Australia (50% year-round reduction; Power et al., 2016) allows for evaluation of responses to experimental drought across continents. We hypothesized that, at both intercontinental and local scales, experimental drought will significantly alter the assembly of microbial communities in grasslands, with bacterial communities being more sensitive than fungi to water limitation (Austin et al., 2004; Clark, Campbell, Grizzle, Acosta-Martìnez, & Zak, 2009). Specifically, we predicted that the relative abundance of bacterial

TABLE 1 Environmental characteristics of study sites

Site name	Code	Grassland type	MAP (mm)	MAT (°C)	pH
Sevilleta National Wildlife Refuge	SEV Black	Desert	242	13.3	8.5
Sevilleta National Wildlife Refuge	SEV Blue	Shortgrass	242	13.3	8.8
Central Plains Experimental Range	SGS	Shortgrass	342	8.6	6.2
High Plains Grasslands Research Center	WYO	Mixed	384	7.6	7.1
Hays Agricultural Research Center	HAYS	Mixed	577	12.0	7.2
Konza Prairie Biological Station	KNZ	Tallgrass	860	12.9	6.4
DRI-Grass Experimental Site	DG	Australian grassland	800	17.0	6.6

MAP, mean annual precipitation; MAT, mean annual temperature.

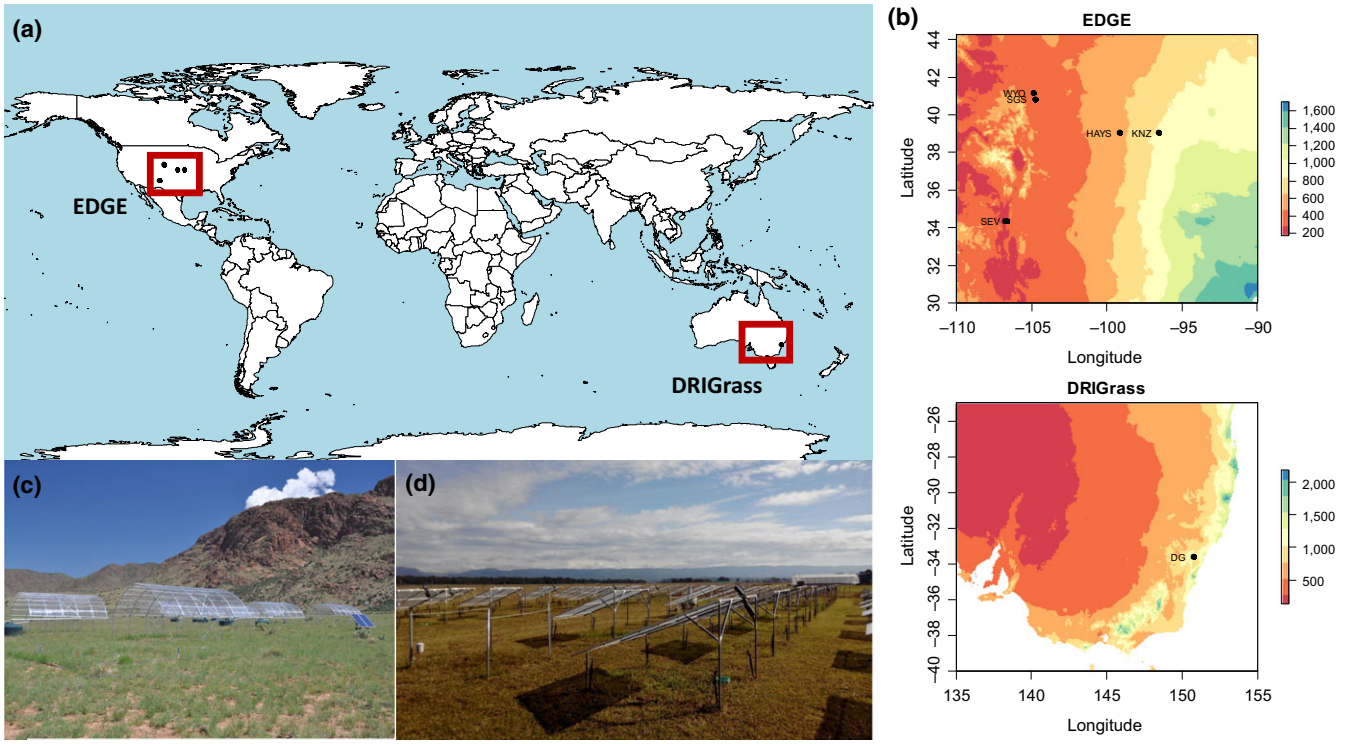

FIGURE 1 (a) Location of study sites; (b) location of sites along the regional precipitation gradient (color scale is in mm); (c) picture of study site at Sevilleta (EDGE); (d) picture of DRI-Grass experimental facility. Site legend is as in Table1

taxa such as Chloroflexi and Actinobacteria, known to be adapted to arid conditions (Acosta-Martínez et al., 2014; Maestre et al., 2015), would increase in response to drought events. In contrast, we predicted an increase in potential enzymatic activity in droughted plots, as a result of enzyme and substrate accumulation during periods of low soil moisture (Austin et al., 2004). An increase in potential enzymatic activity could also be linked to reduced competition with plants for soil resources (Schwinning & Sala, 2004) and/or to greater inputs of organic matter associated with the death of roots and shedding of foliage (Sinsabaugh et al., 2008).

2 | MATERIALS AND METHODS

2.1 | Study sites

The seven sites considered in this study encompass different types of grassland ecosystems and span a wide range of climatic and soil conditions (Table 1; Figure 1). None has been grazed for the last 15 years. Six experimental sites were selected across the Central and Southwest United States along a large precipitation gradient (242–860 mm). These sites are part of the EDGE (Extreme Drought in Grasslands Experiment) experimental platform (http://edge.biology.colostate.edu/). They included desert grasslands, shortgrass and tallgrass prairies, and mixed grasslands. Soil texture varied from sandy to clay loams (Knapp et al., 2015), whereas soil pH ranged from slightly acidic (6.25) at the High Plains Grasslands Research Center, Wyoming, to basic (8.82) at Sevilleta National Wildlife Refuge, New Mexico. Konza Prairie, Kansas, is the only site that is burned annually, whereas the rest have not burned in recent times.

In addition to the six sites in the United States, another study site was selected in Eastern Australia (DRI-Grass; Drought, and Root Herbivore Interactions in a Grassland). The site is a mesic grassland near Richmond, NSW, Australia, at an elevation of 25 m a.s.l. Mean annual precipitation is 800 mm (Australian Government Bureau of Meteorology, Richmond – WSU Hawkesbury Station1). The soil is a Blackendon Sand, with a sandy loam texture and pH of 6.38. The most abundant species include C4 grasses such as *Axonopus fissifolius*, *Cynodon dactylon*, *Cymbopogon refractus*, *Eragrostis curvula*, and *Paspalum dilatatum*, C3 grasses including *Microlaena stipoides* and *Lolium perenne*, and C3 forbs such as *Hypochaeris radicata* and *Plantago lanceolata*. Although the sites encompass different types of grassland ecosystems and span a wide range of edapho-climatic conditions, DRI-Grass is most comparable to the mesic American grassland (Konza; Figure 1).

2.2 | Experimental treatments

The EDGE platform was set up in spring 2013 at Sevilleta and 2014 at the other four sites and uses rainout shelters to impose a drought by reducing each precipitation event by 66% for the entire growing season, the latter varying between sites. This is roughly equivalent to a year-round reduction of 50% precipitation. Each experimental

treatment is replicated ten times at each site. Plots are 3 × 4 m and are hydrologically isolated from the surrounding soil matrix by aluminum flashing installed to varying depth depending on site.

The DRI-Grass experiment started in June 2013 and consists of sixty plots (1.9 × 2.5 m) and five precipitation treatments. Plots are covered with fixed rainout shelters, which exclude ambient precipitation inputs. In this study, we used a subset of two treatments ("ambient" and "reduced amount" – a reduction of 50% compared to ambient) replicated six times, for a total of twelve plots. The shelters have open sides and are covered with UV-transparent Perspex roofs sloped at an angle of 18°. Both treatments involve water re-application through a programmable automated irrigation system. Soil moisture and temperature were continuously recorded using sensors installed in almost all plots. More detailed information on the field site and experimental design can be found elsewhere (Power et al., 2016).

2.3 | Soil sampling

Soils at all sites were collected in 2015 during the main growing season (March in Australia and July in the U.S. sites). Eight to ten soil samples were collected from each plot at a depth of 0–10 cm and bulked. Once in the laboratory, samples were kept at 4°C until further processing within a few days. A small subsample was also immediately frozen at −20°C for soil microbial analyses.

2.4 | Soil properties and microbial extracellular enzyme activity analyses

Gravimetric soil water content (%) was measured after drying a known amount of soil at 70°C and then weighing it. Soil pH was measured using a 1:2.5 ratio of fresh bulk soil to deionized water. Soils were assayed for: β-1,4-glucosidase (BG), and β-D-cellobiohydrolase (CBH) enzymes, involved in the degradation of cellulose and other beta-linked glucans (the major components of plant cell walls), β-1,4-*N*-acetylglucosaminidase (NAG), associated with the degradation of chitin and peptidoglycan (major microbial cell wall components), and phosphatase (PHOS; phosphorus mineralization) for the P cycle. Briefly, assays were conducted by homogenizing 1 g of soil in 30 ml of pH-adjusted 50 mM sodium acetate buffer. The pH of the buffer was adjusted to match the soil pH of each site (Figure 1). The homogenized solutions were then added to a 96-deep-well (2 ml) microplate. Replicate soil slurry controls and 4-methylumbelliferone (MUB) standard curves of 0–100 μm were included in each sample. Fluorometric substrates (Sigma-Aldrich, reference numbers: M3633 for BG, M6018 for CBH, M2133 for NAG, and M8883 for PHOS) were added to soil slurries and then incubated for 1.5 hr at 35°C. Following incubation, the supernatant solution was transferred into corresponding wells in a black, flat-bottomed 96-well plate. The plates were then scanned on a microplate fluorometer using an excitation wavelength of 365 nm and an emission wavelength of 450 nm. Enzyme assays for the Australian samples were carried out on the same day at the Hawkesbury Institute for the Environment, Western Sydney University, Australia using a 2300, EnSpire® Multilabel Reader (PerkinElmer, Boston, MA, USA), whereas samples from all EDGE sites were analyzed on the same day at the University of New Mexico.

2.5 | Quantitative PCR

All molecular analyses were undertaken at the Hawkesbury Institute of the Environment on a subset of three replicates per EDGE site and on all six DRI-Grass replicates. First, we extracted the DNA from each soil sample using the PowerSoil kit (Mo Bio Laboratories, Carlsbad, CA, USA) following the manufacturer's instructions. Soils from DRI-Grass were extracted and stored at −80°C until further analyses, whereas soils from the EDGE sites were extracted at the University of New Mexico, frozen, and then shipped to Australia. Once there, all samples were defrosted and qPCR reactions carried out using 96-well plates. Reactions consisted of 5 μl of polymerase mix, 1 μl of template DNA, 0.3 μl of each primer, and 3.4 μl of H_2O, giving a final volume of 10 μl. Bacterial 16S rRNA gene and fungal internal transcribed spacer (ITS) were amplified with the Eub 338-Eub 518 and ITS 1-5.8S primer sets (Evans & Wallenstein, 2012). The abundance of fungi and bacteria were then expressed as the number of ITS or 16S rRNA gene copies g^{-1} soil, respectively.

2.6 | Amplicon sequencing

DNA samples were analyzed using Illumina MiSeq 2x 301 bp (bacteria) or 2x 280 bp (fungi) paired end sequencing (Caporaso et al., 2012) and the 341F/805R (bacteria) and FITS7/ITS4 (fungi) primer sets (Herlemann et al., 2011; Ihrmark et al., 2012). The quality of all Illumina R1 and R2 reads was assessed using FASTQC (Andrews, 2010), low quality regions ($Q < 20$) were trimmed from the 5′ end of the sequences (0 bp from R1 and 22 bp from R2 for primer set 341F/805R; 5 bp from R1 and 50 bp from R2 for primer set FITS7-ITS4R) using SEQTK (https://github.com/lh3/seqtk). The paired ends were subsequently joined using FLASH (Magoč & Salzberg, 2011). Primers were removed from the resulting sequences using SEQTK and a further round of quality control was conducted in MOTHUR (Schloss et al., 2009) to discard short sequences (<380 bp for primer set 341F-805R; <150 bp for primer set FITS7-ITS4R), as well as sequences with ambiguous characters or more than eight homopolymers. Operational Taxonomic Units (OTUs) were built at 97% sequence similarity using UPARSE (Edgar, 2013). Singletons were discarded, as well as chimeric sequences identified by the UCHIME algorithm using the recommended SILVA gold 16S rRNA gene or UNITE reference databases for bacteria and fungi, respectively (Edgar, Haas, Clemente, Quince, & Knight, 2011). OTU abundance tables were constructed by running the usearch_global command (http://www.drive5.com/). Taxonomy was assigned to OTUs in MOTHUR using the naïve Bayesian classifier (Wang, Garrity, Tiedje, & Cole, 2007) with a minimum bootstrap support of 60% and the Greengenes database version 13_8 (DeSantis et al., 2006; McDonald et al., 2012) for bacteria or the dynamic UNITE version 6 dataset

(Kõljalg et al., 2013) for fungi. The OTU abundance tables were rarefied to an even number of sequences per sample to ensure equal sampling depth (8,115 sequences for bacteria and 34,403 sequences for fungi), prior to calculating alpha diversity metrics using MOTHUR (Schloss et al., 2009).

2.7 | Statistical analyses and numeric calculations

All analyses reported were carried out in R version 3.4.0 (R Core Team, 2017). Enzyme activity data, fungal and bacterial abundance (log-transformed), richness, diversity, and the dominance of all taxa with a mean relative abundance higher than 1% were analyzed using linear mixed-effects models, with drought treatment as the fixed factor and location as a random effect. We also carried out linear models at the site level with experimental treatment as a fixed factor. Analyses were performed using the "lme" and "lm" functions from the NMLE and STATS packages, respectively.

We analyzed changes in the composition and structure of bacterial and fungal communities by means of permutational analyses of the variance (9,999 permutations) using the "adonis" function in VEGAN. Samples were nested within sites using the "strata" argument. Results from the permutational multivariate analyses were visualized by means of two nonmetric multidimensional scaling (NMDS) analyses using fungal and bacterial OTU data. For this, we used the "metaMDS" function of the VEGAN package.

To investigate how microbial responses to drought may change along environmental gradients, we calculated an Effect Size (ES) of each microbial variable considered, including the two first components of the NMDS for bacterial and fungal communities. We defined the ES as the difference between the droughted and control plots for each site. We then carried out nonparametric Spearman-rank correlation analyses ($n = 7$ sites) between the ESs and climate (MAP and MAT) and soil pH. A significant correlation between a predictor variable and the ESs indicates that the magnitude of the response of the dependent microbial variable to drought is proportional to the environmental variable.

To build a more holistic understanding of the responses of bacterial and fungal microbial communities to drought and environmental variation, we carried out structural equation models (Grace, 2006) using the "sem" function from the LAVAAN package. All *a priori* models and citations for all hypothetical paths are depicted and referenced in Figure S9 and Appendix S1, respectively. Microbial activity was the final response variable and was computed as the average of the *z*-score of each individual soil enzyme (i.e., equivalent to the simple multifunctionality index as described in Maestre et al., 2012). In our model, climate (MAP) and drought affected all variables but, given that drought is an experimental treatment, these were independent of each other (i.e., they are exogenous variables). Mean annual precipitation was consistently used as our climatic variable over MAT because we wanted to be able to better predict SWC, which we presumed was a key variable in our model. Exploratory analyses also showed that models considering MAP had a consistently better goodness of fit than models using MAT. Soil water content and pH were hypothesized to have a direct effect on microbial community attributes (structure, abundance, diversity, and richness in separate models) and microbial activity. Finally, microbial community attributes directly influenced microbial activity. Model fit was considered good when the χ^2 test and its associated *p*-value were low (<2) and high (>.05), respectively. The root-mean-square error of approximation (RMSEA) was also used to evaluate the goodness of fit. A model has a good fit when RMSEA is <0.05 and its associated *p*-value is >.05.

3 | RESULTS

Across all sites, bacterial communities were consistently dominated by globally distributed bacterial phyla (Figure 2), including: Actinobacteria (26.1%), Proteobacteria (23.4%), Acidobacteria (18.0%), Planctomycetes (6.6%), Verrocomicrobia (6.4%), Chloroflexi (6.1%), Bacteroidetes (4.5%), Gemmatimonadetes (2.9%), and Firmicutes (1.8%). Dominant fungal taxa included: Ascomycota (55.0%), Basidiomycota (24.5%), Chytridiomycota (1.8%), Glomeromycota (2.8%), and Zygomycota (5.3%). This microbial community composition is similar to that reported for global drylands (Maestre et al., 2015), but contrasts with previous studies in which Acidobacteria and Basidiomycota were found to be the dominant bacterial and fungal phyla, respectively, at the global scale (Ramirez et al., 2014; Tedersoo et al., 2014). Despite common patterns in the relative abundance of the main taxa at the highest taxonomic level (classes/phyla), microbial communities, particularly for fungi, differed widely between sites at the OTU level (Figure 2).

Experimental drought significantly altered the assembly of soil bacterial ($p < .001$) and, to a lesser extent, fungal communities ($p = .090$) across seven sites in two continents (Figures 2, 3 and S1–S5; Table S1). The magnitude of the fungal community response to drought at the species (OTU) level was proportional to the amount of ambient precipitation (Spearman's rho = 0.81, $p = .027$; Figure S6; Table S2); i.e., we found the largest differences between control and droughted plots at the most mesic end of the precipitation gradient, represented by KNZ and HAYS, in the United States, and the Australian site (Figure 2).

Of all taxa with a mean relative abundance greater than 1%, three bacterial phyla (Actinobacteria, Chloroflexi, and Gemmatimonadetes), seven classes (Rubrobacteria, Acidobacteriia, Deltaproteobacteria, Thermoleophilia, Chloroflexi, Actinobacteria, Pedospherae) and one genus (*Rubrobacter*), and one fungal class (Glomeromycetes) were consistently affected by drought ($p < .05$; Figure 2 and Table S1). Glomeromycetes and Gemmatimonadetes decreased, whereas Actinobacteria (genus *Rubrobacter*, in particular) and Chloroflexi, generally described as more dominant in drylands, increased.

In parallel with consistent microbial responses in terms of community assembly and relative abundance of some taxa, other microbial community attributes and taxa responded in a site-dependent manner, thus highlighting the context dependency of some drought

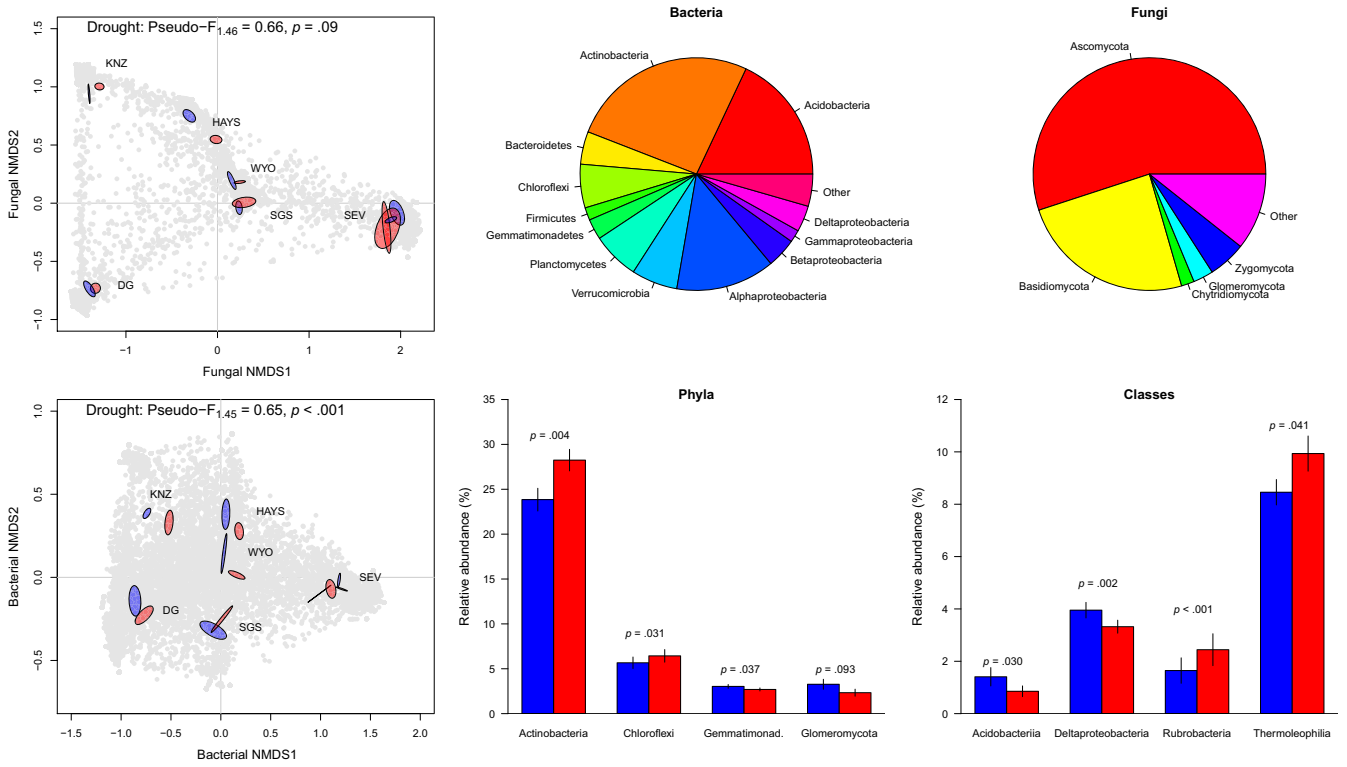

FIGURE 2 Drought effects on microbial community composition and major bacterial and fungal taxa. Gemmatimonad. = Gemmatimonadetes. Blue bars = control; red bars = drought

effects (Figures S1–S5 and Table S2). For example, fungal abundance and richness increased in WYO, whereas fungal and bacterial richness decreased at the driest location (SEV). Analyzed as effect sizes, the relative abundance of Chytridiomycota decreased more at the warmest sites (Spearman's rho = −0.76, p = .049; Figure S6). In contrast, Chloroflexi (Spearman's rho = −0.85, p = .016) and Rubrobacteria (Spearman's rho = −0.81, p = .027) increased in response to drought at the drier sites, whereas Dothideomycetes (Spearman's rho = 0.82, p = .023), Rubrobacteria (Spearman's rho = 0.86, p = .014), and Acidobacteria (Spearman's rho = 0.96, p < .001) were more positively affected at the most acidic sites (Figure S6). Tenericutes (Spearman's rho = 0.87, p = .010) and Chlamydiae (Spearman's rho = −0.76, p = .049) responded more positively and negatively to drought, respectively, toward the wettest end of the precipitation gradient (Figure S6).

Changes in soil microbial community composition in response to drought occurred in parallel with changes in potential microbial enzyme activity, with responses of the latter being strongly site-dependent (Figures 3 and S7; Table S3). For example, two C-degrading enzymes and one N-degrading enzyme increased in Australia in response to drought, whereas the C-degrading enzymes ß-glucosidase and cellobiohydrolase increased in SGS and KNZ, respectively. Similar to fungal community composition, drought sensitivity of enzyme activity was only apparent at the more mesic sites. Potential enzyme activity was also highly significantly related to soil pH at the intercontinental scale (Figures 3 and S8), peaking at neutral pH, results that are in line with previous global studies evaluating soil enzyme relationships with soil pH (Sinsabaugh et al., 2008).

The SEM for the fungal community structure explained 97% of the variance in the first NMDS axis and 89% of potential microbial activity (Figure 3a). Similarly, the SEM for the bacterial community structure explained 94% of the variance contained in the first NMDS axis and 85% of potential microbial activity (Figure 3b). In contrast, models using bacterial and fungal richness, Shannon diversity, and abundance (qPCR) data explained a much lower proportion of the total variance in potential enzyme activity (Figure S10). Indeed, models constructed using the relative abundance of major bacterial and fungal phyla also consistently explained a lower proportion of the variance in microbial activity than OTU-level analyses. In these models, the relationship between soil enzyme activity and microbial relative abundance was, however, significantly positive in the case of Acidobacteria, Verrucomicrobia, Glomeromycota, and Basidiomycota, whereas it was negative in the case of α-Proteobacteria, ß-Proteobacteria, δ-Proteobacteria, Gemmatimonadetes, Zygomycota, and Chytridiomycota (Table S4). As expected, experimental drought reduced soil water content which, in turn, greatly influenced the structure of fungal and bacterial communities, as defined by the NMDS axes. The SEM analyses also indicate a significant increase in fungal richness in response to drought (Figure S10e). In addition, SEMs indicate that the composition of both fungal and bacterial communities is also greatly driven by variations in MAP and soil pH. In contrast, the effect of drought was only statistically significant for

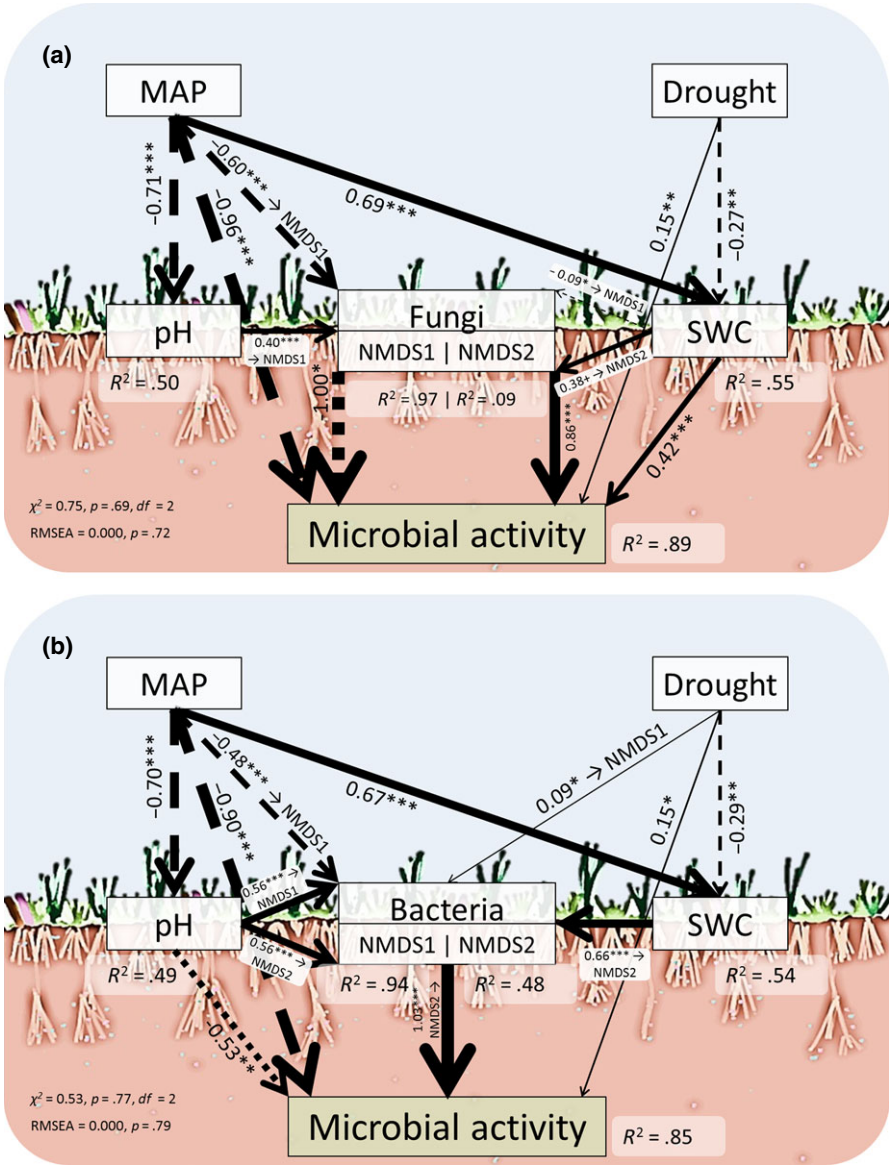

FIGURE 3 Structural equation models depicting the direct and indirect effects of drought and environmental conditions on microbial community composition and activity. (a) Fungal community, represented by the first two axes of the NMDS. (b) Bacterial community, represented by the first two axes of the NMDS. SWC = soil water content. MAP = mean annual precipitation. *$p < .05$; **$p < .01$, ***$p < .001$

bacterial, but not fungal, community composition, findings that support our previous analysis.

4 | DISCUSSION

Our study provides novel experimental evidence that drought is a major climatic driver of the assembly of soil microbial communities. This is in agreement with a previous observational study that suggested that microbial communities are highly responsive to long-term climatic changes such as those from increases in aridity (Maestre et al., 2015). However, our study provides, to the best of our knowledge, the first widespread evidence (i.e., from multiple sites spanning a precipitation gradient and two continents) that the assembly of microbial communities is also highly vulnerable to short-term climatic changes (i.e., 2–3 years of experimentally imposed drought), which may affect the provision of key microbially mediated ecosystem services such as decomposition and nutrient cycling.

The magnitude of the fungal community response to drought at the species (OTU) level was proportional to the amount of ambient precipitation, whereby the largest absolute differences between control and droughted plots were found at the most mesic end of the precipitation gradient, represented by KNZ and HAYS, in the United States, and the Australian site. This suggests a common fungal community response pattern to drought in locations that are thousands of kilometers apart and that show large differences in terms of microbial community composition, as is particularly well-illustrated by the large site separation along the second NMDS axis. This greater microbial sensitivity to drought at more mesic sites contrasts with the generally observed pattern of greater drought sensitivity of aboveground productivity in more arid grasslands (Knapp et al., 2015), which suggests that plant and microbial communities may respond differently along precipitation gradients.

At the higher taxonomic level (phylum and class), some consistent response patterns also emerged. For example, Glomeromycetes and Gemmatimonadetes decreased, whereas Actinobacteria (genus

Rubrobacter, in particular) and Chloroflexi, generally described as more dominant in drylands, increased. These bacterial taxa are highly resistant to desiccation and low resource conditions, which may allow them to outcompete other microbial taxa under extreme drought (Battistuzzi & Hedges, 2009). Particularly relevant was the response of Tenericutes and Chlamydiae, two widely distributed bacterial phyla known for containing species that can cause serious plant and animal diseases, and that responded more positively and negatively to drought, respectively, toward the wettest end of the precipitation gradient. Some studies have suggested an increase in soil pathogenicity under climate change scenarios (van der Putten, Macel, & Visser, 2010) whereas our results suggest more complex, taxa-dependent interactions between altered precipitation regimes and soil-borne pathogens.

We assessed microbial community functioning using a high-throughput analysis of soil extracellular enzyme profiles. Extracellular enzymes decompose soil organic matter and reflect microbial nutrient demand (Sinsabaugh et al., 2008). Changes in soil microbial community composition in response to drought occurred in parallel with changes in potential microbial enzyme activity, with responses of the latter being strongly site-dependent. Similar to fungal community composition, drought sensitivity of enzyme activity was only apparent at the more mesic sites, suggesting that drought-driven alterations in soil microbial communities may further impact the functioning of essential ecosystem services such as nutrient cycling and decomposition, particularly at wetter locations.

The use of SEMs allowed us to build a more holistic understanding of the responses of bacterial and fungal microbial communities and soil functions to experimental drought. In the case of both bacteria and fungi, our SEMs explained an enormous portion of the variation in the distribution of microbial communities and enzyme activities (>85%). In contrast, models using bacterial and fungal richness, Shannon diversity, and abundance (qPCR) data explained a much lower proportion of the total variance in potential enzyme activity. Acidobacteria, Verrucomicrobia, Glomeromycota, and Basidiomycota showed a positive effect on enzyme activities, whereas it was negative in the case of α-Proteobacteria, ß-Proteobacteria, δ-Proteobacteria, Gemmatimonadetes, Zygomycota, and Chytridiomycota (Table S4), highlighting a strong link between microbial community composition and soil enzyme activities. This result further suggests that not all major taxa are equally important for maintaining highly functional grassland soils.

Strikingly, both extreme drought and higher soil water content, the latter mainly explained by MAP (positively) and drought treatment (negatively), enhanced potential microbial activity, suggesting that the effects of long-term and short-term climatic phenomena may operate through different mechanisms. For example, greater enzyme activity may be associated with greater organic matter inputs and rhizosphere activity at the wetter end of the precipitation gradient (Sinsabaugh et al., 2008), but substrate accumulation may drive enzyme response under more droughted conditions (Austin et al., 2004). Greater enzyme activity may also be due to reduced competition with plants, given that the levels at which microbes become water-limited are typically much lower than those for plants (Delgado-Baquerizo, Maestre, Rodríguez, & Gallardo, 2013; Schwinning & Sala, 2004), or to extra organic matter inputs associated with the death of fine roots and shedding of foliage. Striking also was the direct negative link between MAP and microbial activity, which may be due to the fact that most of the positive effects of MAP on microbial activity are indirect (e.g., via increased SWC and variations in soil pH and microbial community composition). In addition, SEM models indicate that the composition of both fungal and bacterial communities is also greatly driven by variations in MAP and soil pH. In contrast, the effect of drought was only statistically significant for bacterial, but not fungal, community composition, findings that support our previous analysis. Taken together, our results suggest that drought-induced changes in soil microbial community composition and structure, rather than changes in abundance and diversity, are likely to have the most important consequences in terms of ecosystem functioning and, therefore, affect the ability of these systems to provide key services on which our societies and economies critically depend.

Our results provide unequivocal evidence that as little as 2–3 years of drought can alter the assembly of microbial communities in grasslands from two continents, with clear implications for ecosystem functioning. In particular, our study reinforces the role of distributed networks of comparable experiments to study the impacts of drought (Fraser et al., 2013; Tielbörger et al., 2014) and unveiled consistent responses in contrasting grassland ecosystems in Australia and North America that share similar climatic and edaphic conditions. In response to drought, we found a greater abundance of drought-resistant bacterial taxa (Actinobacteria and Chloroflexi) and lower abundance of a widely symbiotic, mycorrhizal-forming fungal class (Glomeromycetes). Climate change driven impacts on soil microbial communities were modulated by the local environmental context, including an increase and decrease in the relative abundance of two pathogenic taxa along a gradient of increasing precipitation. However, unlike aboveground responses, many of the belowground variables evaluated (e.g., fungal community composition) exhibited a particularly high degree of resistance to drought at the driest end of the gradient. This pattern suggests that plant and microbial communities may respond differently to drought along precipitation gradients, which opens new questions about the potential role of the disruption or weakening of plant–microbial interactions under climate change scenarios due to a decoupling in the response of both groups.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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