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RESEARCH ARTICLE

Experimental drought re-ordered assemblages of root-associated fungi across North American grasslands

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Abstract

1. Plant-associated fungi can ameliorate abiotic stress in their hosts, and changes in these fungal communities can alter plant productivity, species interactions, community structure and ecosystem processes.
2. We investigated the response of root-associated fungi to experimental drought (66% reduction in growing season precipitation) across six North American grassland ecosystem types to determine how extreme drought alters root-associated fungi, and understand what abiotic factors influence root fungal community composition across grassland ecosystems.
3. Next generation sequencing of the fungal ITS2 region demonstrated that drought primarily re-ordered fungal species' relative abundances within host plant species, with different fungal responses depending on host identity. Grass species that declined more under drought trended toward less community re-ordering of root fungi than species less sensitive to drought. Host identity and grassland ecosystem type defined the magnitude of drought effects on community composition, diversity and root colonization, and the most important factor affecting fungal composition was plant species identity.
4. Fungal diversity was less responsive to drought than fungal composition. Across ecosystems, latitude and soil pH were better predictors of fungal diversity than experimental drought. Drought significantly reduced fungal diversity and evenness only in sideoats grama *Bouteloua curtipendula* and reduced root colonization only in blue grama *B. gracilis*.
5. Our results illustrate that predicting the effects of drought on root-associated fungi will require attention to host plant identity and ecosystem type. By comparing our findings against soil microbe responses in the same experiment, we propose the hypothesis that fungi in plant roots are less sensitive to drought than fungi inhabiting soils. Our study highlights the importance of studying community-level

re-ordering of fungal composition for understanding plant responses to climate change.

KEYWORDS
climate change, dark septate endophytes, diversity, latitudinal gradient, microbiome, mycobiome, mycorrhiza

1 | INTRODUCTION

Plant-associated microbes have critical functions in nutrient cycling, carbon storage and plant community structure (Bell et al., 2014; Hartnett & Wilson, 1999). Microbes alter carbon and nitrogen dynamics in soils and plants (Miki & Doi, 2016; Zhu et al., 2018), and root-associated fungi can improve plant uptake of water and soil nutrients (Sharma et al., 2013; Srinivasan et al., 2012). Root-associated fungi can also increase plant biomass (Siefert et al., 2018; Toju et al., 2018), regulate ecosystem dynamics and improve soil carbon storage (Clemmensen et al., 2013; Wang et al., 2016). Understanding the sensitivity of root-associated fungal communities to drought may improve predictions and management decisions under future climates (Rudgers et al., 2020), which are projected to include more frequent and intense droughts, including more frequent 'extreme' droughts (Cook & Seager, 2013; Cook et al., 2013). Extreme drought is defined as annual precipitation that falls below the 5th percentile of historical precipitation in a region (Hoover et al., 2018).

Knowledge of the responsiveness of root-associated fungi to drought is particularly important for foundation plant species because altered fungal interactions in dominant plants could have large impacts on community structure and ecosystem functioning. First, fungal symbionts can change the outcome of plant competition (Bever et al., 1997; Chung & Rudgers, 2016; Crawford et al., 2019), and so they may shape plant community dynamics. Second, fungi may be more important than bacteria for carbon and nutrient cycling in dryland ecosystems (Rudgers et al., 2018) and can be more resistant than bacteria to drought (de Vries et al., 2018; Ochoa-Hueso et al., 2018; Wang et al., 2017). Thus, understanding fungal responses to drought may be especially critical for predicting future communities under climate change. Third, fungal symbionts could affect plant sensitivity to (or recovery from) drought and thereby influence primary production (Elmi & West, 1995; Gehring et al., 2017; Seema et al., 2019).

Most drought studies have focused on changes in soil microbial communities rather than plant-associated microbes (meta-analysis by Zhou et al., 2018). Plant-associated fungi, in particular, could experience fewer direct effects of drought than soil-dwelling fungi because they are sheltered by their host, or plant-associated fungi could be indirectly affected by drought because the plant host responds to drying (Denison & Kiers, 2011; Millar & Bennett, 2016; Von Rein et al., 2016). For example, across 30 angiosperm species, drought re-ordered sequence abundances of endophytic root bacteria more strongly than rhizospheric bacteria (Fitzpatrick et al., 2018), suggesting that plant-associated microbes may be more sensitive to drought than soil microbes. Mutualistic fungi, in particular, may decrease with drought due to indirect effects such as declines in host plant biomass or in resource subsidies to microbes, potentially compounding negative direct effects of climate change on plant health (Classen et al., 2015; Millar & Bennett, 2016). In one dryland study, ectomycorrhizal fungal communities were reduced to a few dominant species during a natural drought event; however, these taxa were more, not less, beneficial to plants than was the pre-drought community (Gehring et al., 2014). Since some fungi are known to ameliorate the effects of drought on plants (Ganjeali et al., 2018; Johnson et al., 2014; Kivlin et al., 2013; Seema et al., 2019), changes to root-associated fungal composition could feed back to alter plant fitness in future climates. The few tests for drought effects on plant–microbe feedbacks return conflicting results. In one grassland study, drought increased negative feedback, potentially enhancing plant species coexistence (Kaisermann et al., 2017); in two others, drought neutralized feedbacks (Fry et al., 2017; Snyder & Harmon-Threatt, 2019). Thus, understanding drought-induced changes to microbial communities in the rooting zone can help to improve predictions on how drought alters plant–microbe feedback loops that influence primary productivity and plant community composition (Chase, 2007; Crawford et al., 2019; Kaisermann et al., 2017; Lau & Lennon, 2012).

Relatively few studies have tested how experimental drought affects symbiotic fungi in plant roots across multiple study sites and ecosystem types. Although many field experiments have tested drought effects on plant-associated fungi (Bell-Dereske et al., 2017; Cotton, 2018; Deveautour et al., 2018, 2019; Giauque & Hawkes, 2016; Hartman & Tringe, 2019; Hopkins et al., 2018; Karlowsky, Augusti, Ingrisch, Akanda, et al., 2018; Karlowsky, Augusti, Ingrisch, Hasibeder, et al., 2018; Mickan et al., 2019; Motiejunaite et al., 2018; Rasmussen et al., 2020; Staddon et al., 2003; Walter, 2018; Yuste et al., 2011; Zhong et al., 2019), few have included more than one study site, and results are contradictory, especially among ecosystems. For example, in a 4-year experiment manipulating rainfall in a California meadow, arbuscular mycorrhizal (AM) fungal diversity increased under drought conditions (Hawkes et al., 2011). In contrast, a 4-year drought in a subtropical forest showed reductions only in other metrics of AM fungi, such as spore density and root colonization (Maitra et al., 2019). Given the idiosyncratic results of prior studies, progress can be made by disentangling whether divergent results are caused by different methods

or because community responses vary among sites or ecosystem types. Such progress requires cross-site experiments.

Ecosystems may respond differently to drought conditions for several reasons, including their functional composition, successional status and temporal heterogeneity (Grime et al., 2000; Tielbörger et al., 2014). There are two competing hypotheses about variation in resistance to extreme drought among ecosystem types. One hypothesis is that arid communities are more resistant to drought than mesic communities because they are adapted to a history of low precipitation. Alternatively, mesic communities may be more resistant to drought than arid communities because factors other than historical drought exposure, such as specific leaf area or leaf turgor loss point, are key to resistance (Griffin-Nolan et al., 2019; Huxman et al., 2004). Across a precipitation gradient in North American grasslands, primary production and plant composition were more sensitive to drought in arid than mesic grasslands (Knapp et al., 2015), supporting the hypothesis that factors other than historical drought exposure are predictors of drought resistance. However, Ochoa-Hueso et al. (2018) found that soil microbial communities from mesic ecosystems across these same sites were instead *more sensitive* to drought than soil communities from arid ecosystems, suggesting that sensitivity to drought differs among taxonomic subsets of the whole community. Neither of these studies examined microbes directly associated with plant roots, so it is unknown whether these rhizosphere fungi will follow host plant responses or track soil microbe responses to drought.

Our study aimed to fill gaps on the responses of non-AM, root-associated fungi to drought. A recent meta-analysis of microbial responses to altered precipitation recorded 24 studies of soil fungi, but none on root-associated fungi (Zhou et al., 2018). This synthesis revealed that AM fungi increased in soils with precipitation additions (using phospholipid fatty acid assays), although testing whether these fungi also decline in roots requires sampling plant tissue rather than soil. Even in soils, fungal diversity and community composition have not been examined across enough studies for quantitative synthesis (Zhou et al., 2018). While soil samples can capture the AM fungi that are obligately plant-associated, other important fungal rhizosphere members, such as ascomycetes, may occur rarely or at different relative abundances in soil than in roots. Expanding study beyond AM fungi is important because the Ascomycota may actually be more beneficial to plants during drought than under normal levels of water availability (reviewed by Kivlin et al., 2013). As such, we specifically targeted non-AM fungi in roots to help fill this knowledge gap.

In this study, we investigated root-associated fungi in foundation grasses of central North American grasslands using a cross-site experiment that imposed a consistent, severe growing season drought for 2–3 years. To examine how these grasslands differed in the fungi associated with the dominant grasses, and to test how drought affected grassland root-associated fungi, we addressed the following questions: (a) What climatic or edaphic factors most strongly influence root-associated fungal community composition across North American grassland ecosystems? (b) Does extreme drought alter root-associated fungal communities, and how much does the fungal response to drought vary among foundation grass species or grassland ecosystems?

2 | MATERIALS AND METHODS

2.1 | Study sites

The Extreme Drought in the Grasslands Experiment (EDGE) included six grassland sites spanning the central United States, with mean annual precipitation ranging 242–860 mm (Table 1). No cattle grazing occurred during the study, but the northern mixed grass prairie (HPG) and southern mixed grass prairie (HAR) sites had been grazed prior to the experiment. The tallgrass prairie site (KNZ) was burned annually, following local management practices (Collins et al., 1998; Griffin-Nolan et al., 2018).

TABLE 1 List of sites and grass species sampled for Extreme Drought in the Grassland Experiment (EDGE). MAP, mean annual precipitation; MAT, mean annual temperature

Site name	Site code	Latitude	Longitude	Grassland type	MAT (°C)	MAP (mm)	Grass species
Central Plains Exp'l Range	CPR	40.81553	−104.74560	mixed grass	8.6	342	*B. gracilis*
Hays Agricultural Research Center	HAR	38.85861	−99.33538	mixed, short	10.1	654	*B. dactyloides* *B. curtipendula* *B. gracilis* *S. scoparium*
High Plains Grassland Research Center	HPG	41.18246	−104.89707	mixed grass	7.6	384	*B. gracilis*
Konza Prairie	KNZ	39.10077	−96.56309	tallgrass	12.9	860	*A. gerardii* *B. curtipendula* *S. scoparium*
Sevilleta National Wildlife Refuge B	SEVB	34.34214	−106.62261	desert, short	13.3	260	*B. gracilis* *B. eriopoda*
Sevilleta National Wildlife Refuge G	SEVG	34.33731	−106.72839	desert, short	13.3	242	*B. eriopoda*

2.2 | Experimental design

Each site had control and drought plots (6 m × 6 m) arranged in ten spatial blocks, with each block containing one ambient and one drought plot. Growing season drought was imposed using rain-out shelters from mid-April to end of August (Yahdijan & Sala, 2002). Shelters were constructed with transparent corrugated polycarbonate roofs (6 m × 6 m × 3.4 m tall; Stuppy Greenhouse) that excluded 66% of rainfall, imposing drought higher than the 99th percentile of the long-term average (Table S1). Ten control plots per site received ambient precipitation. Drought treatments started in 2013 at SEVB and SEVG and in 2014 at SGS, HPG, HAR and KNZ (Table 1). Each plot was trenched to ~0.2–1 m depending on rooting depth and surrounded by a similar depth of aluminum flashing to prevent lateral surface run-on and subsurface flow.

2.3 | Focal plant sampling

We selected six dominant plant species (Table 1): *Andropogon gerardii* (big bluestem), *Bouteloua curtipendula* (sideoats grama), *B. dactyloides* (buffalograss), *B. eriopoda* (black grama), *B. gracilis* (blue grama) and *Schizachyrium scoparium* (little bluestem). At each site, roots from one focal plant individual from each plot were excavated with a transplanting shovel in 2015, two (SGS, HPG, HAR, KNZ) or three (SEVB, SEVG) years after the start of the drought. Some focal species did not occur in some plots due to the extent of their natural geographic ranges, resulting in an unbalanced design at a few sites (Table S1). To control for seasonal variation in plant–microbe interactions, we collected roots at similar phenological stages across sites. We used NOAA climate data nearest to each site to determine growing degree days (GDD) over March–October with a base temperature of 0°C, the minimum growth temperature for grasses (Henebry, 2013). Then, we sampled each site at similar GDD values (M = 2,656°C ± 351 *SD*) to control for the phenological stage and target sampling at peak growing season when drought treatment effects on soil moisture would be strong, but plants would not be senescing.

Roots from each plant were placed into a sterile Whirl-Pak (Nasco), shipped on ice overnight to Western Illinois University and processed within 48 hr. In the laboratory, twenty live roots were washed in DI water to remove soil particles and dead roots, cut into 1-cm fragments with a surface-sterilized scalpel, mixed and separated into three fractions: (a) a fraction for sequencing was frozen at −80°C; (b) a fraction for microscopy was stored in plastic tissue cassettes (Slimsette; Simport) in 50% ethanol and (c) a fraction was immediately surface-sterilized for culturing (J.A. Rudgers, S. Fox, A. Porras-Alfaro, J. Herrera, C. Reazin, D.R. Kent, L. Souza, Y.A. Chung, & A. Jumpponen, unpubl. data).

The percentage cover of each focal plant species was recorded twice each growing season to capture early season (April/May) and late season (August/September) species composition. We estimated aerial cover of each plant species in four 1 m × 1 m quadrats per plot. Mean cover of each grass species across the four quadrats in each plot was calculated to give cover values per square metre at the plot level for early and late season measures.

2.4 | Edaphic factors

Five edaphic variables were analysed for each EDGE site: ammonium, nitrate, phosphorus (total P), soil pH, soil organic matter (SOM) and soil gravimetric water content. We sampled 10–20 g of soil from beneath plants outside of the EDGE plots to capture natural edaphic conditions. Samples were pooled for each grass species × site combination. Phosphorus and soil pH were determined using protocols in (Robertson, 1999), while SOM was determined using loss on ignition (Zhang & Wang, 2014). Inorganic nitrogen concentration was determined colorimetrically using a Lachat Autoanalyzer QuikChem 12-107-06-1-A and 12-107-04-1-F.

2.5 | Climate factors

We created climate variables for 2015 by extracting climate data at the 800-m spatial resolution using the PRISM database (PRISM Climate Group, 2019). We calculated growing degree days for March–October using a base temperature of 0°C and annual precipitation (mm). We also determined average growing degree days and annual precipitation for the 30-year average up to and including year 2015.

2.6 | Fungal root colonization microscopy

We removed roots from cassettes and prepared them for microscopy following methods described in Herrera et al. (2010); this method focuses on colonization by ascomycetes. Five roots from each of the five randomly selected plants of each species were selected based on presence of root hairs, and the lack of pathogenic lesions or physical damage. Each field of view was overlaid with 10 equidistant intercepts using a virtual reticle and scored for melanized hyphae, melanized vesicles, microsclerotia, hyaline hyphae, hyaline vesicles and spores using a variation of the line intercept method on images taken at 200X (Herrera et al., 2010, methods detailed in Supplemental Methods). Root colonization was calculated as the proportion of ten equidistant intercepts crossing any fungal structure.

2.7 | Next generation sequencing

Using the MoBio PowerSoil DNA Isolation Kit (MoBio), total genomic DNA was extracted from twenty root segments (1 cm) per plant then stored at −20°C until PCR amplification. Extracted DNA was quantified spectrophotometrically (ND2000; NanoDrop Technologies) and standardized to 1.0 ng/μl for PCR (White et al., 1990). We targeted the fungal Internal Transcribed Spacer 2 (ITS2) of the ribosomal RNA gene for amplification because of its interspecific variability as described in (Blaalid et al., 2013), using primers fITS7 (5′-GTGARTCATCGAATCTTG-3′; Blaalid et al., 2013) and ITS4

(5′-TCCTCCGCTTATTGATATGC-3′; Ihrmark et al., 2012). These primers target non-AMF sequences. Detailed methods on PCR reactions are provided in Supplemental Methods. Illumina primers and adapters were added using NEBNext® DNA MasterMix for Illumina (New England Biolabs Inc.) at the Integrated Genomics Facility (Kansas State University). Libraries were paired-end sequenced using MiSeq Reagent Kit v3 (Illumina) with 2 × 300 cycles in a combined run. Raw paired-end sequence data are available at the Sequence Read Archive (SRA) at the National Center for Biotechnology Information (NCBI) under BioProject PRJNA545269 and BioSamples SRR9182406–SRR9182603.

2.8 | Bioinformatics

Sequence data were processed using mothur (version 1.38.1; Schloss et al., 2009). Sequences in paired-end.fastq files were contiged into a total of 4,778,262 total reads; those with any ambiguous bases, any mismatches to MIDs, more than one mismatch to primers, or with homopolymeric regions longer than 8bp were removed. The remaining 2,598,063 sequences were truncated to the length of the shortest remaining high-quality reads (228 bp) to facilitate preclustering of near identical reads (Huse et al., 2010) and subsequent OTU clustering with VSEARCH (Rognes et al., 2016) of the unaligned sequence dataset. The near identical reads, which differed by ≤2 bp, were clustered (Huse et al., 2010) to minimize platform-generated errors, and the remaining sequences were further screened for putative chimeras (UCHIME; Edgar et al., 2011), which were removed, resulting in 2,485,397 reads. Unique reads were assigned to taxonomic affinities using the Naive Bayesian Classifier (Wang et al., 2007) and the UNITE INSD (http://unite.ut.ee/repository.php) as a reference. Reads without a taxon affinity on the Kingdom level were removed from the dataset. Data were clustered to OTUs using VSEARCH (Rognes et al., 2016) at 97% sequence similarity. OTUs were assigned to taxonomic affinities based on the previous unique sequence assignments. To improve data integrity, we omitted rare OTUs with sequence count ≤10 (Brown et al., 2015; Oliver et al., 2015). The final dataset contained 2,469,957 reads, with mean reads per sample of 34,305 ± 52,958 (*SD*).

To estimate richness, diversity and composition, data were rarefied to 10,000 reads per individual plant to avoid biases resulting from sequence yields (Gihring et al., 2012). In mothur (Schloss et al., 2009), we iteratively calculated Good's coverage (i.e. ratio of local OTU singletons to total number of sequences in a sample), OTU richness (S_{obs}), the complement of Simpson's diversity ($1 - D$: $1 - \sum p_i^2$) and Simpson's evenness (E: $1 - \sum p_i^2 / S_{obs}$), with p_i representing the frequency of each OTU in a sample.

2.9 | Statistical analysis

To test for drought effects on the percentage cover of focal grass in 2015, we analysed maximum cover (either early or late season, depending on the grass species) with a linear mixed effects model for each plant species × site. Block was included as a random effect (R package NLME; Bates et al., 2015). To test for the relationship between fungal community composition re-ordering (difference between drought and control NMDS centroids for each site × species group) and plant effect size (absolute value of *RII* for change in plant cover caused by drought), we used a generalized least squares model (R package NMLE; Bates et al., 2015).

2.9.1 | What climatic or edaphic factors most strongly influence root-associated fungal community composition across grassland ecosystems?

We evaluated the relative importance of alternative abiotic factors in predicting fungal diversity and abundance using model selection procedures. Each candidate model had one of the following abiotic predictors: latitude, soil pH, soil nitrate, soil gravimetric water content, soil total phosphorus, 30-year growing degree days or 30-year mean precipitation. All candidate models included the drought treatment, the random effect of plant species and the abiotic predictor × drought treatment interaction. We ranked the relative importance of abiotic predictors using the second order Akaike information criterion (AICc); we analysed each predictor separately and ranked them in order to avoid multicollinearity among climate and edaphic variables (Anderson, 2008). We also determined marginal R^2 (R package PIECEWISESEM; Lefcheck, 2016). Model selection procedures were also performed individually for *B. gracilis*, because it had the largest representation across sites due to its large geographic range.

2.9.2 | Does extreme drought alter root-associated fungal communities, and how much does the fungal response to drought vary among foundation grass species or grassland ecosystems?

We analysed treatment effects on the proportional abundance matrix of rarefied OTUs with perMANOVA (Primer 6.1.10; Clarke & Gorley, 2015). Proportions were calculated after rarefying to aid in interpretation. The model included treatment (drought/control), site (six levels), the random effect of block (nested in site), plant species (six levels), site × treatment and species × treatment. We could not explore site × treatment ×species interactions because the same grass species were not present across all sites (Table S1). The effects of plant species present in only one site (*A. gerardii*, *B. dactyloides*) are partially conflated with site effects (Table 1). We visualized fungal composition using two-dimensional non-metric multidimensional scaling (NMS) with 500 restarts using Bray–Curtis distance (Clarke & Gorley, 2015). All NMS solutions ($k = 2$) had stress of ~0.2. Centroids (*SE*) were plotted in Sigmaplot v14 (Systat Software). We compared dispersion, the divergence in composition among samples for drought versus control groups within each site × plant species combination using PermDISP (Clarke & Gorley, 2015).

Indicator species analysis identified taxa that contributed most to compositional differences among treatments, sites and grass species (R package LABDSV; SIMPER; Clarke & Gorley, 2015; Dufrene & Legendre, 1997; Roberts, 2016). We presented the five best indicators for drought over all sites and species, as well as the five best indicators for drought within each grass species. For each indicator, we also calculated the relative interaction intensity index (*RII*) from mean proportional abundance data as (drought−control)/(drought + control) (Armas et al., 2004). Negative *RII* indices indicate taxa that declined under drought, whereas positive *RII* indices indicate increases. Five indicators for each category were chosen because the cumulative contribution of OTUs in the indicator analysis dropped off sharply after ~5 taxa, which was also reflected by the *RII* for the top five indicator OTUs. Thus, the five best indicators were those most likely to contribute meaningfully to fungal composition.

We analysed Shannon diversity, richness, Chao richness and Simpson evenness of rarefied fungal OTUs as well as total root colonization (%) with linear mixed effects models with the fixed effects of treatment (drought/control), site, plant species, site × treatment, species × treatment and the random effect of block (nested in site; R package LME4; Bates et al., 2015). Pairwise comparisons of estimated marginal means (R package EMMEANS; Lenth et al., 2019; R v.1.1.163; R Core Team, 2019) decomposed effects with >2 levels.

To link fungal with plant responses, we analysed the magnitude of the change in focal plant species percentage cover for each species × site combination (*RII*) as a function of the distance between drought and control centroids for fungal community composition (see distance matrix, perMANOVA, above) using generalized least squares. A nonlinear model fit the data similarly to a linear model using the second order AICc (ΔAICc = 0.05; Bartoń, 2013). However, nonlinearity better represented the relationship graphically. Models were created in package NLME (Pinheiro et al., 2016), and the final model was $RII_{plant} \sim 0.605 \times \exp(-0.017 \times \text{Centroid distance}_{fungi})$.

3 | RESULTS

3.1 | General patterns in root-associated fungal composition

Of ~2.5 million high-quality reads and 1,205 OTUs, most OTUs belonged to phyla Ascomycota (62%), Basidiomycota (23%) or Glomeromycotina (11%). Basal lineages of fungi represented <2% of all OTUs. Fewer than 2% of OTUs could not be classified beyond kingdom Fungi. Representation across grass species was uneven due to the natural abundances of plants in EDGE plots: *A. gerardii* (11% of total OTUs found for 85 individual OTUs, 20 plots), *B. curtipendula* (14%, 104 OTUs, 25 plots), *B. dactyloides* (5%, 65 OTUs, 10 plots), *B. eriopoda* (15%, 60 OTUs, 28 plots), *B. gracilis* (42%, 207 OTUs, 77 plots) and *S. scoparium* (13%, 95 OTUs, 24 plots).

Within Ascomycota, the most common fungal phylum in our dataset, 27% of OTUs were classified into order Pleosporales, 15% were unclassified Ascomycota, 10% were order Chaetothyriales and 10% were order Sordariales, with other groups each representing <7% of Ascomycota OTUs. Within Basidiomycota, Agaricales represented 43% of OTUs, unclassified Agaricomycetes were 10%, Cantharellales were 7% and the rest were distributed among 23 groups, most comprising <2% of OTUs. Forty percent of sequences were identified to genus level. Just eleven genera were present in >1% of total sequences (Figure 1). These most common genera were *Rhizophagus* (12%; Glomerales), *Podospora* (4%; Sordariales), *Lophiostoma* (3%; Pleosporales), *Preussia* (3%), *Cladophialophora* (2%), *Marasmius* (2%), *Lachnum* (2%), *Marchandiomyces* (2%), *Mortierella* (2%), *Chaetomium* (2%) and *Monosporascus* (2%). To represent the differences in fungal composition between treatments and site × species groups, we plotted the proportional abundance (number of reads/total reads per sample) of these eleven most abundant genera, averaged for each plant species × site × treatment group (Figure 1).

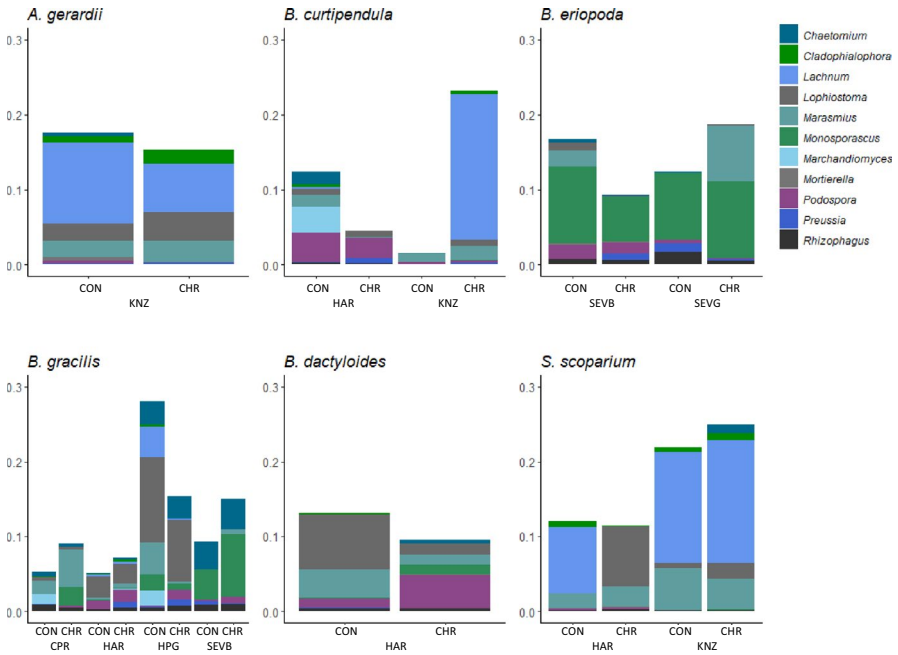

FIGURE 1 Stacked bar plots of average proportional abundance of most abundant 11 fungal genera for each grass species × site × treatment combination

3.2 | Host plant responses to drought

Among the focal host plant species at each site, most grasses had declined in cover in response to chronic drought at the time that we sampled plant roots (Figure 2). *Bouteloua eriopoda* declined most strongly in response to drought (96% lower cover in drought than control at SEVG and 93% lower cover in drought than control at SEVB; Figure 2a,b), followed by *B. gracilis* (81% lower cover in drought than control at SEVB, 35% lower in drought than control at CPR, 24% lower in drought than control at HPG; Figure 2b–e). By 2015, *B. dactyloides*, *B. curtipendula* and *S. scoparium* had declined under drought, but not significantly (Figure 2e,f), and cover by *A. gerardii* increased slightly under drought (Figure 2f).

3.3 | What climatic or edaphic factors most strongly influence root-associated fungal community composition across grassland ecosystems?

Given that site was a significant predictor of root-associated fungal community composition (see Table 3), we explored site-level climate and edaphic factors as predictors of fungal diversity, colonization and composition. In general, all environmental covariates correlated with the Shannon diversity (H') and Chao richness of fungal communities, while evenness and root colonization correlated with fewer edaphic factors (Table 2). Latitude ($p < 0.05$) and soil pH ($p < 0.01$) were strong correlates of all measures of fungal community composition (Table 2), but the strongest abiotic predictors of the root-associated fungal community differed among the community metrics we examined. Across all grass species and sites, latitude was the most important predictor of Shannon diversity and Chao richness: both increased at higher latitudes (Table 2; both $p < 0.05$). However, soil pH (ranging from 6.2 to 8.3 among ecosystems) was the most important predictor of fungal evenness, which declined with increasing pH. Growing degree days was the most important predictor of root colonization, with more colonization at warmer sites (Table 2). We also examined environmental correlates of root-associated fungal community composition within *B. gracilis* for which we had the largest number of replicates across EDGE sites. Similar results were obtained as for all plant species, apart from evenness being better predicted by latitude than by soil pH (Table 2).

3.4 | Does extreme drought alter root-associated fungal communities, and how much does the drought response vary among grassland ecosystems or foundation grass species?

3.4.1 | Fungal community composition

Drought had a small, but statistically significant, influence on fungal composition on average across all grassland ecosystems and plant species (Table 3; perMANOVA: $p = 0.0036$). Plant species identity and ecosystem type (site effect) had stronger effects on fungal

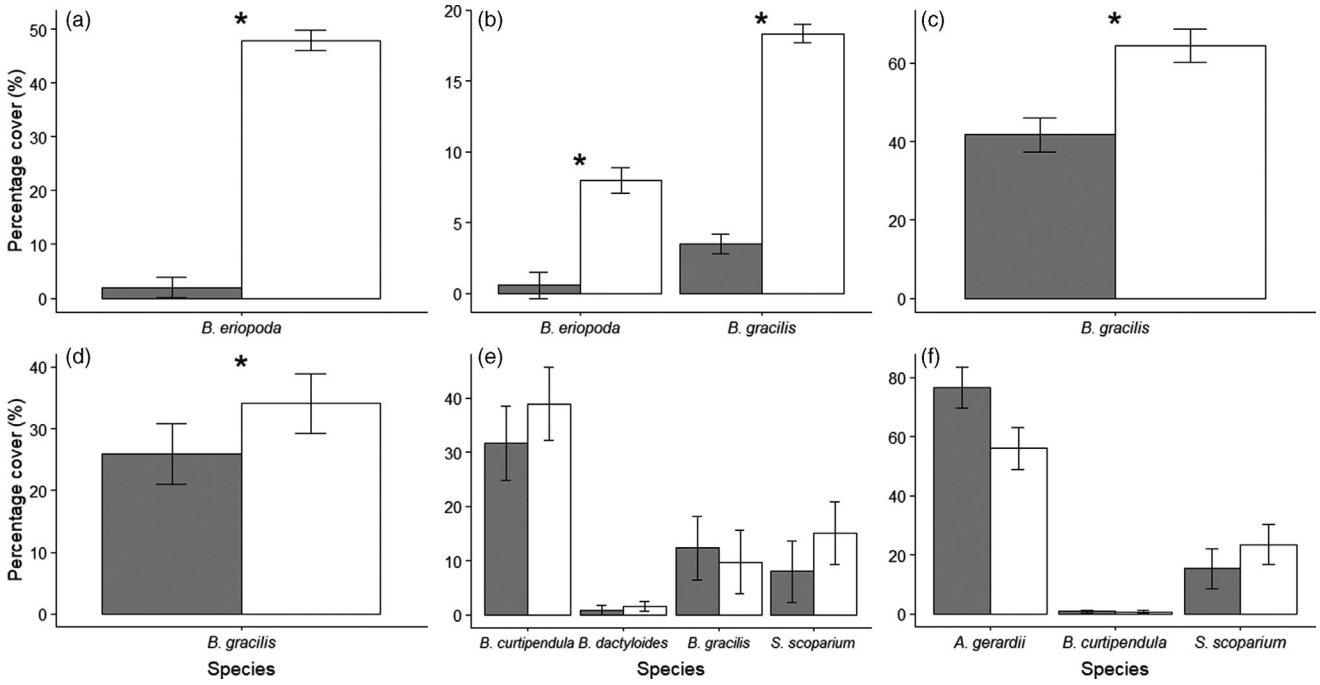

FIGURE 2 Percentage cover of focal grass taxa in control plots (white bars) and in response to EDGE chronic drought (dark bars) in 2015 at each of the six sites: (a) SEVG, (b) SEVB, (c) CPR, (d) HPG, (e) HAR, (f) KNZ. Bars show mean plant cover (%) per plot ± *SE* after averaging values for four (1 m × 1 m) quadrats sampled per plot. '*' indicate cases in which plant species cover significantly changed with chronic drought in 2015

TABLE 2 Environmental covariate analysis results for root-associated fungal community structure (Shannon Diversity Index, Chao richness, Evenness or Root colonization) across all EDGE sites and host plant species. Bold text indicates covariates that were the best predictors of community structure based on model selection procedures using AICc. GDD is growing degree days, and PPT is mean annual precipitation, both determined over a 30-year window

Environmental covariate	R^2	ΔAICc	β	p
Shannon Diversity Index				
Latitude	**0.36**	**0.00**	**7.19**	**<0.0001**
Soil pH	0.20	27.17	−5.67	<0.0001
Soil nitrate	0.08	52.07	2.76	0.0058
Soil gravimetric water content	0.09	50.71	2.84	0.0045
Soil phosphorus	0.12	42.06	−3.34	0.0008
GDD 30	0.27	226.57	−5.60	<0.0001
PPT 30	0.12	47.12	3.56	0.0004
Chao richness				
Latitude	**0.20**	**0.00**	**5.43**	**<0.0001**
Soil pH	0.05	40.14	−2.08	0.0378
Soil nitrate	0.13	33.95	3.03	0.0024
Soil gravimetric water content	0.18	24.59	3.74	0.0002
Soil phosphorus	0.11	24.82	−3.67	0.0002
GDD 30	0.17	18.71	−4.03	0.0001
PPT 30	0.15	32.42	3.23	0.0012
Evenness				
Latitude	0.04	3.36	2.00	0.0459
Soil pH	**0.07**	**0.00**	**−3.00**	**0.0027**
Soil nitrate	0.04	3.48	−0.09	0.9296
Soil gravimetric water content	0.05	1.95	−0.47	0.6419
Soil phosphorus	0.02	7.12	−0.67	0.5046
GDD 30	0.03	5.10	−1.74	0.0827
PPT 30	0.04	2.91	0.38	0.7032
Root colonization				
Latitude	0.20	12.07	−4.61	<0.0001
Soil pH	0.16	11.02	4.35	<0.0001
Soil nitrate	0.01	34.70	0.88	0.3795
Soil gravimetric water content	0.01	34.43	1.01	0.3128
Soil phosphorus	0.09	21.41	3.66	0.0002
GDD 30	**0.24**	**0.00**	**5.82**	**<0.0001**
PPT 30	<0.01	35.16	0.38	0.7035

composition than experimental drought (Figures 3 and 4, Table 3; perMANOVA: $p < 0.0001$). However, interactions between treatment × site or treatment × species were not statistically significant (Table 3; perMANOVA: $p > 0.05$). Of the five best indicator OTUs for the drought treatment across all ecosystems and plant species,

TABLE 3 Results of perMANOVA to examine the response of root-associated fungal composition to drought treatment, focal plant species identity and site. The model included the main effects of treatment, site and plant species as well as the interaction effects of treatment × site and treatment × species. *p*-values ≤ 0.05 are shown in bold

Factor	df	F	p
Treatment	1	0.68	**0.0036**
Site	5	9.56	**<0.0001**
Species	5	2.96	**<0.0001**
Treatment × Site	5	2.47	0.1369
Treatment × Species	5	2.45	0.1796

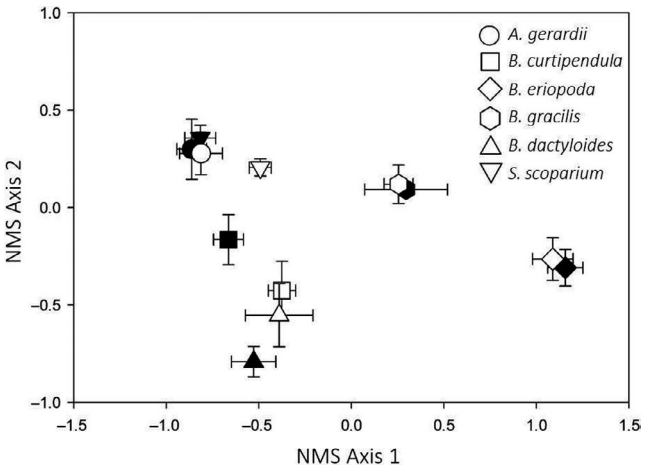

FIGURE 3 Non-metric multi-dimensional scaling plot of the centroid ± *SE* of axis values of root-associated fungal community composition for six plant species comparing EDGE chronic drought (black) versus control (white) plots. Data include all sites. Species significantly differed (perMANOVA $p < 0.0001$), and drought differed from control on average across species ($p = 0.0036$), but species identity did not interact with the drought treatment ($p = 0.1796$). NMS two-dimensional stress = 0.25

three were Ascomycota and two were Basidiomycota (Table S2). The strongest indicator of the drought treatment across species and ecosystems was an unclassified Marasmiaceae (Agaricales), for which sequence abundance increased 200% under drought (Table S2).

Both grass species and ecosystem types diverged in root-associated fungal community composition (Figures 3 and 4). The largest overall differences in community composition occurred between *B. eriopoda* and *S. scoparium* (perMANOVA; similarity = 0.63), whereas the fungal communities of *A. gerardii* and *S. scoparium* were the most similar (perMANOVA; similarity = 13.83, Figure 3). Grassland ecosystem types also diverged in root-associated fungal community compositions (Figure 4). The largest differences between sites were between KNZ and SEVB (perMANOVA; similarity = 0.46). SEVB and SEVG, the sites most proximal geographically, were the most similar in composition (similarity = 6.33).

Overall fungal community composition was not significantly affected by drought within single sites or plant species in individual

perMANOVA (Table S5; Figure 5; perMANOVA: $p > 0.05$ for all comparisons; $p < 0.1$ for *B. gracilis* and *S. scoparium*). Additionally, drought did not alter fungal community dispersion for any individual site or species (Figure 5; permDISP, $p > 0.05$). However, specific individual fungal taxa responded strongly to drought within plant species and ecosystems. For example, in *B. gracilis*, the most important drought indicator was an unclassified Marasmiaceae (Agaricales), which increased by ~220% (Table S3). Similarly, within *B. gracilis*, the proportional abundance of *Lachnum* decreased with drought in site HPG ($p = 0.048$; Figure 1). Within *B. eriopoda*, *Lophiostoma* proportional abundance decreased in drought plots in site SEVB ($p = 0.02$) but did not change in site SEVG ($p > 0.05$; Figure 1). For *B. curtipendula*, proportional abundance of *Lachnum* increased in drought plots in site KNZ ($p = 0.002$; Figure 1). Within *S. scoparium*, drought caused *Lophiostoma* proportional abundance to increase in site HAR ($p = 0.02$) and across all sites together ($p = 0.02$). Plant species present in only one site, *A. gerardii* and *B. dactyloides*, had no changes to the most abundant eleven genera due to drought (both $p > 0.05$; Figure 1). Across the thirty indicator OTUs for drought within each grass species (five per focal grass species), 20% were Basidiomycota and 80% were Ascomycota (Table S2). Across the thirty indicator OTUs for drought within sites (five per site), 70% were Ascomycota and 30% were Basidiomycota (Table S4).

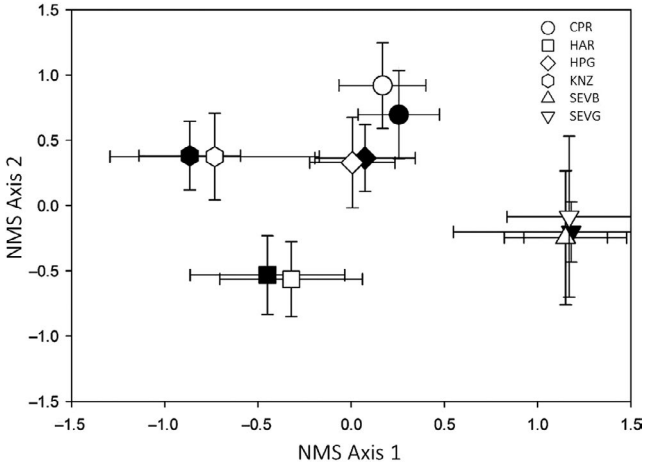

FIGURE 4 Non-metric multi-dimensional scaling plot of centroids ± *SE* of axis values for each EDGE site comparing chronic drought (black) versus control (white) plots. Data include all plant species. Sites significantly differed in root-associated fungal community composition (perMANOVA, $p < 0.0001$), and drought differed from control across sites ($p = 0.0036$), but site did not significantly interact with the drought treatment ($p = 0.1369$). NMS two-dimensional stress = 0.25

3.4.2 | Linking fungal and plant responses

Given that drought re-ordered the relative abundances of fungal taxa, we linked this response to the plant cover response during the same year of observation. The magnitude of responsiveness of plant species to drought (absolute *RII* for percent cover in response to drought) tended to decrease as the change in fungal community

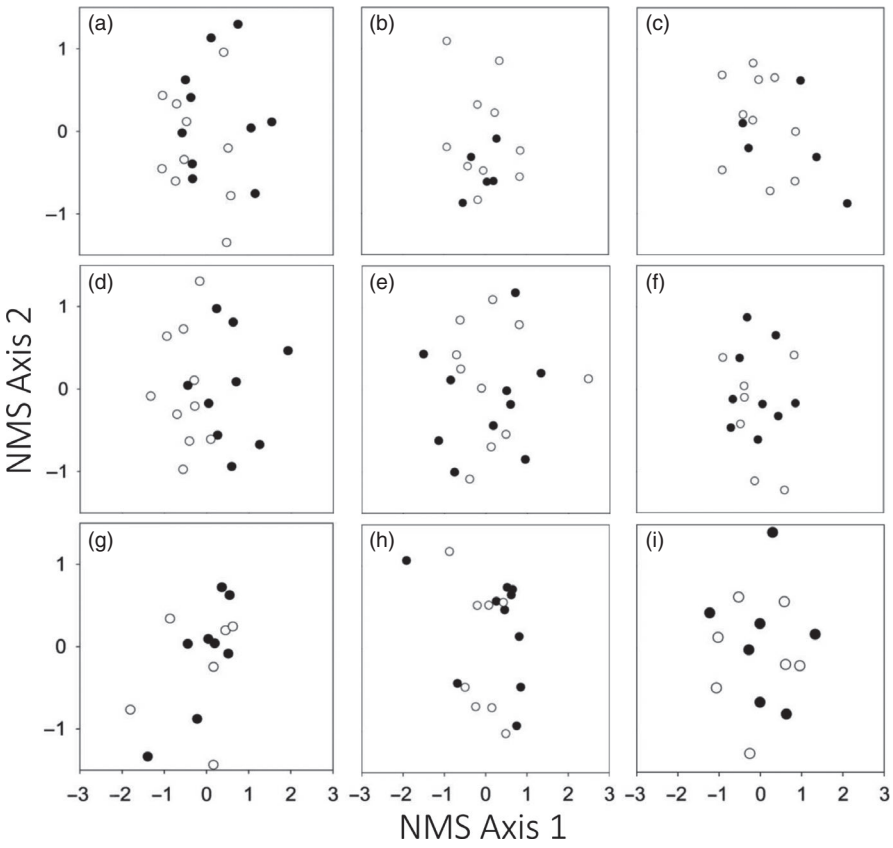

FIGURE 5 Non-metric multi-dimensional scaling analysis of each EDGE site and grass species combination comparing chronic drought plots (black) against control (white) plots. (a) CPR *Bouteloua gracilis* (perMANOVA: pseudo-$F = 0.99$, $p = 0.46$) (b) HAR *B. curtipendula* (pseudo-$F = 1.09$, $p = 0.29$), (c) HAR *B. gracilis* (pseudo-$F = 1.15$, $p = 0.17$), (d) HPG *B. gracilis* (pseudo-$F = 1.30$, $p = 0.10$), (e) KNZ *Andropogon gerardii* (pseudo-$F = 0.79$, $p = 0.80$), (f) KNZ *Schizachyrium scoparium* (pseudo-$F = 0.79$, $p = 0.80$), (g) SEVB *B. eriopoda* (pseudo-$F = 1.30$, $p = 0.14$), (h) SEVB *B. gracilis* (pseudo-$F = 1.32$, $p = 0.14$), (i) SEVG *B. eriopoda* (pseudo-$F = 0.61$, $p = 0.94$). NMS two-dimensional stress ranged from 0.11 to 0.21 across plots

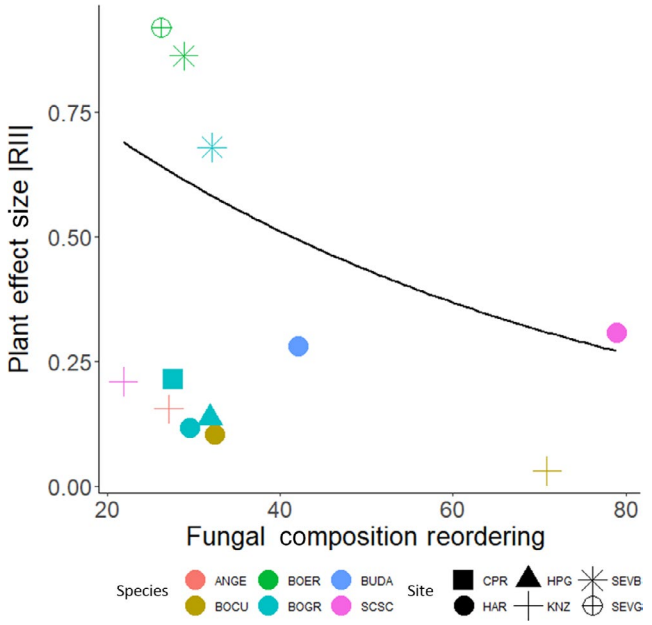

FIGURE 6 Plot of the absolute value of plant response to drought (*RII* for the difference in percentage cover between drought and control) against the difference between drought and control centroids of fungal community composition. Each point represents a plant species (colours) × site (symbols) combination. The best nonlinear model was $RII_{plant} \sim 0.605 \times \exp(-0.017 \times \text{Centroid distance}_{fungi})$, $p = 0.22$

composition increased, although this trend was not statistically significant ($p > 0.05$; Figure 6).

3.4.3 | Fungal diversity

On average across sites and plant species, fungal diversity, richness and evenness did not decline with chronic drought (Table 4). However, some metrics of fungal diversity declined with drought for one plant species (Table 4). Specifically, chronic drought significantly reduced root-associated fungal diversity and evenness in *B. curtipendula* (Table S6; $p < 0.01$) but did not significantly affect any other grass species or site (Tables S6 and S7; $p > 0.05$).

All fungal diversity and abundance metrics differed among EDGE ecosystem types, indicating sufficient statistical power to detect possible effects of drought on fungal diversity (Table 4; $p < 0.05$). Root-associated fungal communities were most diverse at HPG (mean Shannon $H' \pm SE$; 2.84 ± 0.12) and least diverse at SEVG (1.49 ± 0.18). Other sites fell in between: KNZ (2.53 ± 0.11), HAR (2.49 ± 0.07), CPR (2.49 ± 0.12), SEVB (1.76 ± 0.11). Diversity in part reflected differences among sites in the number of dominant grass species present. In Kansas sites (HAR, KNZ), 3–4 foundational grass species occurred in EDGE plots, but only one foundation grass species was present at CPR and SEVG (Figure 2). Grass species differed in root-associated fungal Shannon diversity (Table 4; $p < 0.01$). Chao richness and Simpson evenness followed similar trends but were not significantly different among grass species (Table 4; $p < 0.1$). *B. curtipendula* had the least diverse root fungal community (1.99 ± 0.11, 2 sites). In contrast, *B. eriopoda* had the most diverse fungal community (mean Shannon $H' \pm SE$, 2.67 ± 0.11, 2 sites), even though the site where it dominated (SEVG) was the least diverse site. This occurred because fungal diversity was much greater for *B. eriopoda* at SEVB where it co-dominated with *B. gracilis*, than at SEVG where *B. gracilis* was absent. Diversity of other grass species' root-associated fungi fell in between: *A. gerardii* (2.19 ± 0.17, 1 site), *B. gracilis* (2.21 ± 0.06, 4 sites), *S. scoparium* (2.24 ± 0.15, 2 sites), *B. dactyloides* (2.31 ± 0.15, 1 site).

TABLE 4 Results of ANOVA using linear models to examine responses of root-associated fungal diversity, richness, evenness and root colonization. Models included the main effects of site, treatment and species as well as the interaction effects of treatment × species and treatment × site. Reduced models are shown when interactions were non-significant. *p*-values ≤ 0.05 are shown in bold, *p*-values ≤ 0.07 shown in italics

Factor	*df*	*F*	*p*
Shannon Diversity Index			
Site	5	15.45	**<0.01**
Treatment	1	0.71	0.40
Species	5	5.34	**<0.01**
Treatment × Species	5	2.47	**0.03**
Chao richness			
Site	5	18.64	**<0.01**
Treatment	1	0.25	0.62
Species	5	2.85	**0.02**
Evenness			
Site	5	2.88	**0.02**
Treatment	1	0.55	0.46
Species	5	2.14	*0.06*
Treatment × Species	5	2.08	*0.07*
Root colonization			
Site	4	12.23	**<0.01**
Treatment	1	0.46	0.50
Species	5	7.63	**<0.01**

3.4.4 | Fungal colonization of roots

On average across sites and plant species, fungal root colonization was not altered by drought (Table 4). However, sites differed in levels of root colonization, indicating sufficient statistical power to detect drought effects had they occurred (Table 2; $p < 0.01$). KNZ had the greatest total colonization ($M \pm SE$; 45.98 ± 7% of intercepts). HPG had the least (1.50 ± 8%). Other sites were intermediate: SEVB and SEVG binned together (41.22 ± 7%), HAR (34.35 ± 5%), and CPR (2.35 ± 8%). Grass species also differed in levels of root colonization (Table 4; $p < 0.01$). *B. gracilis* had the greatest colonization (mean % colonization ± *SE*, 49 ± 3%), *A. gerardii* the least (7 ± 1%), perhaps

because roots were instead dominated by AM fungi that were not the focus of our methods. The other grasses had intermediate levels of colonization: *S. scoparium* (26 ± 9%), *B. curtipendula* (25 ± 9%), *B. dactyloides* (23 ± 8%), *B. eriopoda* (20 ± 6%). Root colonization was significantly reduced by drought only in *B. dactyloides* (Table S6, $p = 0.02$) and not at any EDGE site (Tables S6 and S7; $p > 0.05$). However, of all the plant species, *B. gracilis* had the largest drought-induced decline in root colonization, a 7% decline averaged over all sites where it occurred (Table S6; $p = 0.42$).

4 | DISCUSSION

4.1 | What climatic or edaphic factors most strongly influence root-associated fungal community composition across grassland ecosystems?

Our cross-site analysis did not find a strong influence of mean annual precipitation on root-associated fungal communities, for which composition correlated more strongly with soil pH and growing degree days than precipitation (Table 2). Across all sites, latitude, soil pH and average growing season length were important environmental covariates of fungal diversity and composition in grass roots, but site-level precipitation was not (Table 2). Trends included increasing fungal diversity at northerly latitudes and increasing evenness at lower soil pH (Table 2). These trends contrast against typical latitudinal diversity gradients (Hillebrand, 2004) and diverge from the effect of regional precipitation on *soil* fungi revealed in Ochoa-Hueso et al. (2018). However, the relative importance of edaphic and climatic predictors to fungal evenness was similar between our study (Table 2) and that of Ochoa-Hueso et al. (2018). It is important to note that both root-associated and soil fungal results are limited in geographic extent because only six sites were sampled across central North America.

Our exploration of environmental correlates requires the caveat that we could only detect influences from environmental factors we measured and within the geographic range covered by the EDGE sites. In our broader survey of the same grass species across replicated latitudinal gradients over the North American plains (J.A. Rudgers, S. Fox, A. Porras-Alfaro, J. Herrera, C. Reazin, D.R. Kent, L. Souza, Y.A. Chung, & A. Jumpponen, unpubl. data), 26%–37% of variation in root-associated fungal community composition was predicted by environmental covariates such as soil pH, which also covaries globally with soil bacteria composition (Fierer & Jackson, 2006; Lauber et al., 2009). The broader latitudinal survey of root-associated fungi spanned 24 sites, within which the six EDGE sites were embedded (J.A. Rudgers, S. Fox, A. Porras-Alfaro, J. Herrera, C. Reazin, D.R. Kent, L. Souza, Y.A. Chung, & A. Jumpponen, unpubl. data). Both broad and narrow surveys similarly demonstrated the primacy of host plant identity in shaping fungal composition and abundance (Tables 3 and 4; Figure 3). Both studies showed that patterns in grasslands do not follow typical latitudinal diversity gradients with poleward declines (J.A. Rudgers, S. Fox, A. Porras-Alfaro, J. Herrera, C. Reazin, D.R. Kent, L. Souza, Y.A. Chung, & A. Jumpponen, unpubl. data). Other studies of fungal diversity have found that some clades of fungi, notably ectomycorrhizal (EM) fungi, also do not follow typical latitudinal diversity gradients and instead exhibit the greatest diversity where EM fungi experience high speciation rates, which are stimulated by favourable climatic and edaphic conditions as well as by abundant host plants (Sánchez-Ramírez et al., 2015; Tedersoo et al., 2014). As root-associated fungi include ectomycorrhizal clades, the observed lack of a latitudinal diversity gradient for root-associated fungi in EDGE sites may be caused by similar mechanisms. In EDGE sites, we detected some fungal taxa with latitudinal clines in relative abundance. Most notably, Pleosporales had poleward increases, and Hypocreales had poleward declines (J.A. Rudgers, S. Fox, A. Porras-Alfaro, J. Herrera, C. Reazin, D.R. Kent, L. Souza, Y.A. Chung, & A. Jumpponen, unpubl. data). Overall results from EDGE sites supported these broader geographical patterns.

The absence of a single, strong abiotic correlate of root-associated fungal community composition may occur because many fungi in roots occupy multiple ecological niches (e.g. saprobes, endophytes, pathogens), they can break down diverse and complex molecules, and they grow under a variety of conditions (Porras-Alfaro & Bayman, 2011). The wide variety of ecological roles of root-associated fungi may also explain why, in contrast to many other organisms, fungi appear to be less sensitive to climate disruptions (Wang et al., 2017). The sensitivity of the plant and fungal community as a whole can depend on the sensitivity of individual members. For example, context-dependency of fungal responses to severe drought was demonstrated by Jassey et al. (2018), who reported that ecosystem-level respiratory function depended on the drought response of both individual plant and fungal species. Thus, in our study, the drought response of key fungal indicator taxa could have influenced the resistance of the entire root-associated fungal community as well as the sensitivity of the plant hosts to drought (Figure 2).

4.2 | Does extreme drought alter root-associated fungal communities, and how much does the fungal response to drought vary among foundation grass species or grassland ecosystems?

Our analysis of root-associated fungal responses to extreme drought across six North American grassland ecosystems showed that while fungal diversity and abundance in roots were largely unaffected by drought, fungal community composition was significantly reordered. These compositional changes differed both among grass species and among grassland ecosystem types (Figures 3 and 4). Community re-ordering through changes in species rank abundances has been proposed (Smith et al., 2009) and recently documented (Collins et al., 2020; Jones et al., 2017) to be a common response of plant communities to environmental change. Re-ordering of species relative abundances may commonly precede changes in species richness and wholesale turnover in species presence/absence (Jones et al., 2017), although re-ordering of fungal communities has received little attention thus far. In our study, re-ordering of root

fungal communities occurred in all plant species and ecosystem types, despite dramatic declines in dominant grasses at some sites, but not at others (Figures 1 and 2). This result highlights a contrast between the direct (large plant declines) versus indirect (fungal re-ordering) community responses to extreme drought.

Interestingly, the grass species that declined most strongly and directly following EDGE drought (Figure 2, *B. eriopoda*, *B. gracilis*) had among the smallest overall re-ordering of fungal composition (Figures 1 and 3), although the response relationship across all sites and species was not statistically significant (Figure 6). Grass species responses to drought in our study varied depending on drought-related traits, including specific leaf area, leaf nitrogen content and leaf turgor loss point (Anderegg et al., 2016; Griffin-Nolan et al., 2019; Soudzilovskaia et al., 2013). Thus, the dominant grasses differed in their ability to tolerate chronic drought, which could influence (or be influenced by) the responses of their root-associated fungi. Perhaps large re-ordering of root-associated fungal species enabled some plant species to tolerate drought via shifts in fungal community structure, and plants that lacked this flexibility were hit harder, in loss of cover, by the imposed drought.

By imposing the same drought manipulation and sampling methodology across sites, our study revealed strong variation in root-associated fungal communities among plant species and ecosystem types, but consistently low responsiveness of root-associated fungi to drought. These effects were not confounded by the differing methodologies that complicate the inferences that can be made from single-site studies, which form the bulk of the current literature (e.g. Hawkes et al., 2011; Maitra et al., 2019; Mickan et al., 2019). We showed that root-associated fungal community composition, diversity and abundance were not strongly affected by drought, and across all metrics of root fungal community structure, root fungi differed more among ecosystem types and grass species than due to experimental drought. Our finding that root-associated fungal diversity and abundance were largely unaffected by drought across grass species and ecosystems helps to frame contrasting results from previous studies. For example, AM fungal diversity and abundance have been shown to either increase or decrease due to drought in different ecosystems (e.g. Hawkes et al., 2011; Maitra et al., 2019). If the directionality of the response of the root fungal community to drought is both plant species- and site-specific, as is supported by our results, then it follows that studies of different plant species or sites will commonly return opposite findings. Next steps would include seeking generalizations about fungal responses to drought based on plant and soil microbial traits, life histories or ecosystem characteristics; however, more datasets will be required to achieve such generalization (Grigulis et al., 2013; Rudgers et al., 2020).

Our results suggest the new hypothesis that fungi in plant roots are less sensitive to drought than fungi sampled in soils. Plant-associated fungi in our study responded less to drought than total soil fungi sampled at the same EDGE sites during the same summer (Ochoa-Hueso et al., 2018). Although drought significantly altered root-associated fungal community composition in our study (Table 3; Figures 3 and 4), fungal diversity and abundance were unaffected, and drought effects on composition were weaker than other factors, such as host species identity (Figure 3; Table 3; Table S4). In contrast, Ochoa-Hueso et al. (2018) found that EDGE drought altered the community composition of both soil fungi and bacteria, and that changes in soil fungal communities, although weaker than bacterial responses, were proportional to the natural precipitation gradient across sites, with less sensitivity at drier EDGE sites. Soil fungi may be more sensitive to drought than root-associated fungi because changes in soils reflect not only shifts in plant–fungal interactions, but also losses of host plant cover under drought (e.g. Figure 2). Alternatively, root-associated fungi may be less sensitive than soil-inhabiting fungi because plant hosts indirectly shield fungi from drought stress (Wang et al., 2017). However, contradicting our hypothesis that root-associated microbes are less sensitive to drought than soil microbes, a greenhouse drought experiment demonstrated greater sensitivity of bacterial root endophytes than bacteria in soil (Fitzpatrick et al., 2018).

Both saprotrophs and endophytes were represented among the drought-responsive fungal taxa in our study, although more than half (63%) of drought indicator species remained unclassified to a level useful for predicting their ecology or natural history. Our use of the fungal ITS2 region biased our dataset against detection and taxonomic resolution of arbuscular mycorrhizal (AM) fungi, which are more commonly studied by targeting the small subunit rRNA (SSU rRNA) gene (Opik et al., 2010), so our results are exclusive of this functional group. Across all sites and grass species, 74% of identifiable indicator species were inferred to be saprobes or parasites, whereas 26% were taxa known to be endophytes in other systems. The indicator species most responsible for divergence in root-associated fungal communities between drought and control treatments included close relatives of *Crinipellis scabella*, *Lachnum* sp. and *Phialocephala* sp. CM16s1. *Crinipellis* species are common plant pathogens in many ecosystems including grasslands (Singer, 1943), and *C. scabella* increased in abundance under drought. In contrast, two fungal endophytes, *Lachnum*, a putative endophyte found in several plant species with possible antibacterial effects, and *Phialocephala* sp. CM16s1, commonly found in conifer needles, decreased under drought (Bizabani & Dames, 2015; Korkama-Rajala et al., 2008; Stadler et al., 1993; Tanney et al., 2016). While these observations may suggest that beneficial endophytes become less abundant in drought conditions while parasite abundance increases, the effects of drought were complex and context-dependent. For example, the effect of drought on *Lachnum* depended on grass species identity and site. Within *B. gracilis*, *Lachnum* had significantly greater proportional abundance in control plots than drought in site HPG, while for *B. curtipendula*, it had significantly greater proportional abundance in drought plots in site KNZ (Figure 1). Reduced endophyte relative abundance was also evident in roots of *A. gerardii* and *S. scoparium* at the KNZ site, where these two grasses dominated. Meanwhile, the candidate parasite, *C. scabella*, decreased in abundance in *A. gerardii*, *B. gracilis* and *S. scoparium*, but increased in *B. curtipendula* and *B. dactyloides* at the CPR and HAR sites. Given

these complexities, it is not possible to generalize fungal responses based on their putative functional group assignments (e.g. beneficial endophytes vs. pathogens) from other studies because these taxa behaved differently under drought depending on the host plant or grassland ecosystem. We suggest that the functional role of the same fungal taxon may depend strongly on host identity as well as ecosystem type (context-dependency), requiring much richer fungal databases than currently exist (e.g. FunGuild, FunFun; Nguyen et al., 2016; Zanne et al., 2019) in order to assign functional roles to plant-associated fungal taxa.

5 | CONCLUSIONS

In summary, our examination of non-AMF root-associated fungal communities in dominant grassland plant species reinforced the importance of host identity, latitude and edaphic factors in predicting fungal composition, diversity and colonization of roots. While drought altered root fungal community composition by re-ordering species relative abundances, fungal diversity and colonization were mostly resistant to drought. The grass species exhibiting the largest declines under drought had the least re-ordering among root-associated fungi. Thus, re-ordering of the root-associated fungal community might provide plants with an important stabilizing mechanism in response to altered precipitation. Our results suggest that, under predicted future climates, the composition of root-associated fungi in central and western North American grasslands will be more dependent on the edaphic context and grass species identity than on the direct effects of extreme drought on individual members of root fungal communities. The largest changes in plant-associated fungi will likely be caused by shifts in plant relative abundance or plant species loss, rather than by direct responses of plant-associated fungi to climate variables. The complexity of root-associated fungal communities highlights the importance of studying community-level re-ordering for regulating plant responses to climate change.

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AUTHORS' CONTRIBUTIONS

S.L.C., A.K.K. and M.D.S. conceived and built the EDGE experiment; J.A.R., A.J., A.P.-A., Y.A.C. and J.H. conceived ideas for microbial sampling and designed methodology; L.E.B. and M.D.S. and A.J., Y.A.C., A.P.-A. and J.H. led data collection for plants and fungi respectively; D.L., L.E.B., A.J. and J.A.R. analysed the data; D.L. and J.A.R. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The data are archived in the in the US NSF-sponsored EDI repository: environmentaldatainitiative.org. The DOI for the dataset is https://doi.org/10.6073/pasta/f530f48c02d152590057d20febe47e31 (Lagueux, 2020).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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