Received: 19 December 2020 | Revised: 3 August 2021 | Accepted: 7 August 2021

DOI: 10.1111/jbi.14260

Journal of Biogeography WILEY

RESEARCH ARTICLE

Biogeography of root-associated fungi in foundation grasses of North American plains

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Funding information
National Science Foundation

Handling editor: Rosemary Gillespie

Abstract

Aim: Roots and rhizospheres host diverse microbial communities that can influence the fitness, phenotypes, and environmental tolerances of plants. Documenting the biogeography of these microbiomes can detect the potential for a changing environment to disrupt host-microbe interactions, particularly in cases where microbes buffer hosts against abiotic stressors. We evaluated whether root-associated fungi had poleward declines in diversity, tested whether fungal communities in roots shifted near host plant range edges, and determined the relative importance of environmental and host predictors of root fungal community structure.

Location: North American plains grasslands.

Taxon: Foundation grasses – *Andropogon gerardii*, *Bouteloua dactyloides*, *B. eriopoda*, *B. gracilis*, and *Schizachyrium scoparium* and root fungi.

Methods: At each of 24 sites representing three replicate 17°–latitudinal gradients, we collected roots from 12 individuals per species along five transects spaced 10 m apart (40 m × 40 m grid). We used next-generation sequencing of ITS2, direct fungal culturing from roots, and microscopy to survey fungi associated with grass roots.

Results: Root-associated fungi did not follow the poleward declines in diversity documented for many animals and plants. Instead, host plant identity had the largest influence on fungal community structure. Edaphic factors outranked climate or host plant traits as correlates of fungal community structure; however, the relative importance of environmental predictors differed among plant species. As sampling approached host species range edges, fungal composition converged in similarity among individual plants of each grass species.

Main conclusions: Environmental predictors of root-associated fungi depended strongly on host plant species identity. Biogeographic patterns in fungal composition suggested a homogenizing influence of stressors at host plant range limits. Results predict that communities of non-mycorrhizal, root-associated fungi in the North American plains will be more sensitive to future changes in host plant ranges and edaphic factors than to the direct effects of climate.

KEYWORDS
Ascomycota, climate change, dark septate endophytes, distance decay, diversity, foundation species, latitudinal gradient, microbiome, mycobiome, rhizobiome

1 | INTRODUCTION

Understanding the diversity and distribution of host-associated microbes over broad spatial scales may improve predictions of macro-organism biogeography and associated ecosystem functions (Steidinger et al., 2019). Microbiomes can strongly influence host fitness and population dynamics (reviewed by Bass et al., 2019; Brader et al., 2017; Brunel et al., 2020) and have cascading effects on plant community structure and ecosystem biogeochemical processes (Crawford et al., 2019; Fry et al., 2019; Pugnaire et al., 2019; Shi et al., 2019). These extended phenotypes may be especially important for microbiomes associated with foundation plant species, which define community structure and modulate fundamental ecosystem functions (Ellison et al., 2005).

Resolving the biogeography of microbiomes can improve predictions on ecological responses to environmental change that inform conservation and management decisions (Brunel et al., 2020; Tedersoo et al., 2020), particularly in cases where microbes buffer hosts against stressors (reviewed by Kivlin et al., 2013; Porter et al., 2020). For example, failure to include the microbiome in predictive models could overestimate host sensitivity to environmental change if microbial interactions ameliorate host stress (Allison et al., 2010). Alternatively, if future environments disrupt host-microbe interactions, ignoring microbiomes could underestimate sensitivity to change (Pugnaire et al., 2019; Rudgers et al., 2020). Documenting the distributions of host-associated microbes in the context of climatic and edaphic variables can detect the potential for a changing environment to disrupt host-microbe interactions (Glynou et al., 2016; Ranelli et al., 2015; Steidinger et al., 2020; Vetrovsky et al., 2019). Detection requires gradient studies that span the geographic ranges of known host taxa.

Primary constituents of the plant microbiome are microfungi that grow asymptomatically near, on, or inside roots (Philippot et al., 2013). Root-associated fungal taxa include conidial or sterile Ascomycota, often with melanised hyphae that grow inter- and intracellularly in roots (Porras-Alfaro & Bayman, 2011; Sanchez Marquez et al., 2012). These fungi can rival arbuscular mycorrhizal fungi in abundance, particularly in grasses (Herrera et al., 2010; Mandyam & Jumpponen, 2005), but their ecology has been far less studied (Egidi et al., 2019; Knapp et al., 2012). Current evidence suggests that some plant-associated Ascomycota confer resistance, tolerance, or resilience to stress (Herrera et al., 2011; McLellan et al., 2007; Newsham, 2011). Meta-analyses revealed that plant-associated fungi conferred greater stress tolerance than bacteria (Porter et al., 2020) and that root-associated Ascomycota provided the greatest drought amelioration among plant-associated fungal groups (Kivlin et al., 2013).

The biogeography of non-mycorrhizal root fungi has rarely been studied (reviewed by Brunel et al., 2020; Kivlin et al., 2017) relative to other plant-associated microbes (but see Glynou et al., 2018, 2018). For example, latitudinal gradients in the diversity of root-associated Ascomycota are undescribed. In addition, latitudinal gradients in the diversity of *soil* fungi are conflicting. One global analysis demonstrated equatorial peaks in soil fungal richness, and no influence of plant diversity (Tedersoo et al., 2014). In contrast, a more recent analysis documented low tropical soil fungal diversity, with the highest diversity at high latitudes (Vetrovsky et al., 2019). A review on the global biogeography of belowground microbes reported no consistent latitudinal trends for soil fungi (Hendershot et al., 2017). However, global and large regional studies (e.g. Steidinger et al., 2020; Tedersoo et al., 2014; Vetrovsky et al., 2019) rarely sequence fungi from root tissues, unless they target mycorrhizal fungi. While these broad studies reveal important patterns in global biodiversity, narrower gradient studies, constrained to the geographic distributions of host taxa, enable the separation of biogeographic patterns in fungal diversity from gradients in plant diversity. For example, if root fungi are highly host-specific, then root fungal diversity would peak at low latitudes simply because plant diversity is greatest near the equator, not because fungal diversity per host species has a latitudinal pattern. A separate analysis of fungal taxonomic or plant tissue-specific functional groups may help to resolve microbial biogeographic patterns, especially if host identity is an important filter on microbial community assembly (van der Linde et al., 2018; U'Ren et al., 2012; Yang et al., 2019). For instance, in a unique study on mustard plants, *Microthlaspi* spp., across Europe, root fungal community composition varied with latitude (but richness did not), and climatic, soil and spatial factors explained 12% of fungal community similarity (Glynou et al., 2018). Therefore, both global studies that ignore host identity and regional studies that constrain host identity are needed to accurately fill current knowledge gaps.

Grasslands make good candidates for biogeographic studies on the microbiomes of foundation host plants. For example, grasslands cover ~40% of the contiguous land area in the US and provide important ecosystem services (Pendall et al., 2018). Relative to other ecosystems, grasslands are more sensitive to climate (e.g. Heisler-White et al., 2009), setting the stage for microbial mediation of abiotic stress. In North America, plains grasslands are dominated by a core group of long-lived C_4 grasses (Collins & Glenn, 1991) that host non-mycorrhizal and mycorrhizal fungi (Mandyam & Jumpponen, 2008). Grass resource acquisition is regulated in part by root fungi, particularly in arid grasslands, where Ascomycota dominate roots, and arbuscule-forming Glomerales are rare (Green et al., 2008; Porras-Alfaro et al., 2008).

We characterised biogeographic patterns in the root-associated fungi of five foundation grasses of the North American plains. Our study is the first, to our knowledge, to survey non-mycorrhizal root fungi across multiple grassland species and replicated latitudinal gradients (for soil fungi, see Chen et al., 2017). A gradient approach can capture more natural, multifactor environmental contexts than experiments, which may underestimate effect sizes and miss effects on species distributions (Ibanez et al., 2013). General ecological patterns have yet to be documented for non-mycorrhizal fungi in dominant grasses: Are there latitudinal gradients in diversity? What environmental variables best predict fungal composition, and how much do host plant species delimit the environmental drivers of root fungi? Such knowledge lays the groundwork for understanding the ecological roles of non-mycorrhizal fungi in plant roots and predicting community composition under future environmental change.

Here, we addressed the following questions: *(1) Does the diversity, composition, or abundance of non-mycorrhizal root-associated fungi vary with latitude, and are latitudinal gradients similar across host plant species?* We expected a poleward decline in diversity, typical of many taxa, but additionally predicted strong influences of host plant identity, similar to results for other host-associated microbes (e.g. Chalmandrier et al., 2019; Kivlin et al., 2017; Wagner et al., 2016). *(2) Does the composition of non-mycorrhizal root-associated fungi converge near host range edges?* Recent studies have made a strong case for the importance of species interactions at range edges (Louthan et al., 2015; Lynn et al., 2019); however, most host-microbe studies have not evaluated patterns in microbiome composition at range margins. We predicted a homogenization of microbial community composition near host latitudinal range edges due to the increasingly deterministic influence of range-edge stressors, such as temperature and desiccation thresholds, relative to stochastic processes in community assembly (Chase, 2007). Low plant genetic diversity at range edges could also drive high fungal community similarity. An alternative hypothesis is that microbiome composition becomes more stochastic as the relative abundance of the host dwindles at its range limit. We sampled plant species approaching either their northern or southern range limits. *(3) What is the relative importance of climate, edaphic factors, or host traits as predictors of root-associated fungal community structure?* We hypothesised that edaphic factors would rival or exceed host traits or climate, given prior work on soil pH as a key driver of soil microbes (Fierer & Jackson, 2006; Glassman et al., 2017; Lauber et al., 2009; Tedersoo et al., 2020). However, a prevailing influence of edaphic factors is not a foregone conclusion for non-mycorrhizal root fungi. Recent global surveys revealed stronger climatic than edaphic correlates for both soil Ascomycota (Egidi et al., 2019) and all soil fungi (Vetrovsky et al., 2019), and new experimental work indicated a strong role for plant traits in shaping rhizosphere mycobiomes (Sweeney et al., 2020).

2 | MATERIALS AND METHODS

2.1 | Foundation grasses

We targeted dominant, native grasses that span major tribes of Poaceae: *Andropogon gerardii* Vitman (big bluestem), *Bouteloua dactyloides* (Nutt.) J.T. Columbus (buffalograss), *B. eriopoda* (Torr.) Torr. (black grama), *B. gracilis* (Willd. ex Kunth) Lag. ex Griffiths (blue grama) and *Schizachyrium scoparium* (Michx.) Nash (little bluestem).

2.2 | Field sampling

We sampled 24 sites over three, replicate latitudinal transects (Figure 1a) that spanned an east-west gradient of 14° longitude and ~1000 mm in mean annual precipitation, a north-south gradient of 17° latitude (~1430 km, 42°–29°N) and ~10°C in mean annual temperature, and approached a latitudinal range limit for each grass species (either the northern or southern range edge, Figure S1). Sites included all major grassland types: desert, shortgrass, mixed-grass and tallgrass and diverse ecoregions (Figure 1a, Table S1; Rudgers et al., 2021).

We collected 12 individuals per grass species per site. Individuals were selected as the nearest plant every 10 m along five transects spaced 10 m apart (40 m × 40 m grid). To match phenology, we used the nearest National Climatic Data Center station (ncdc.noaa.gov/cdo-web) to determine growing degree days (GDD, 30-year normal) for March–October with a 0°C base for grasses (Henebry, 2013). Then, we timed each field collection to a similar GDD (Table S1). All species did not occur at every site because our gradients transcended host range edges ($N = 624$ plants, Table S1).

2.3 | Root processing

Roots from each plant were placed into a sterile Whirl-Pak (Nasco, Fort Atkinson, WI, USA) and shipped overnight to Western Illinois University (Macomb, IL). Roots were processed within 48 h by cleaning in DI water and using sterile forceps to remove dead roots. We cut live roots into 1 cm fragments with a sterile scalpel and divided fragments randomly into three fractions. A fraction for sequencing was frozen at −80°C. A fraction for microscopy and traits was stored in plastic tissue cassettes (Slimsette, Simport, Beloeil, QC, Canada) in 50% ethanol. A fraction for culturing was immediately surface sterilised (see Appendix S1, *Fungal cultures*).

2.4 | Edaphic variables

We collected 10–20 g soil below each plant, then combined soils into a single sample for each grass species × site combination. We assessed five edaphic variables: ammonium (NH_4^+) and nitrate (NO_3^-) with the Lachat Autoanalyzer QuikChem method 12-107-06-1-A and 12-107-04-1-F (Loveland, CO), soil organic matter (SOM) via loss on ignition (Zhang & Wang, 2014) and phosphorous (total P) and pH following Robertson et al. (1999).

2.5 | Climate variables

We measured soil gravimetric water content (GWC) for each soil sample. We also used daily climate data at 800 m spatial resolution (PRISM Climate Group, 2019) to calculate average GDD and mean annual precipitation as 30-y normals for each site.

2.6 | Plant traits

Trait measurements followed Perez-Harguindeguy et al. (2013). Large values of specific leaf area (SLA) and specific root length (SRL) reflect large plant investment in resource acquisition; small values

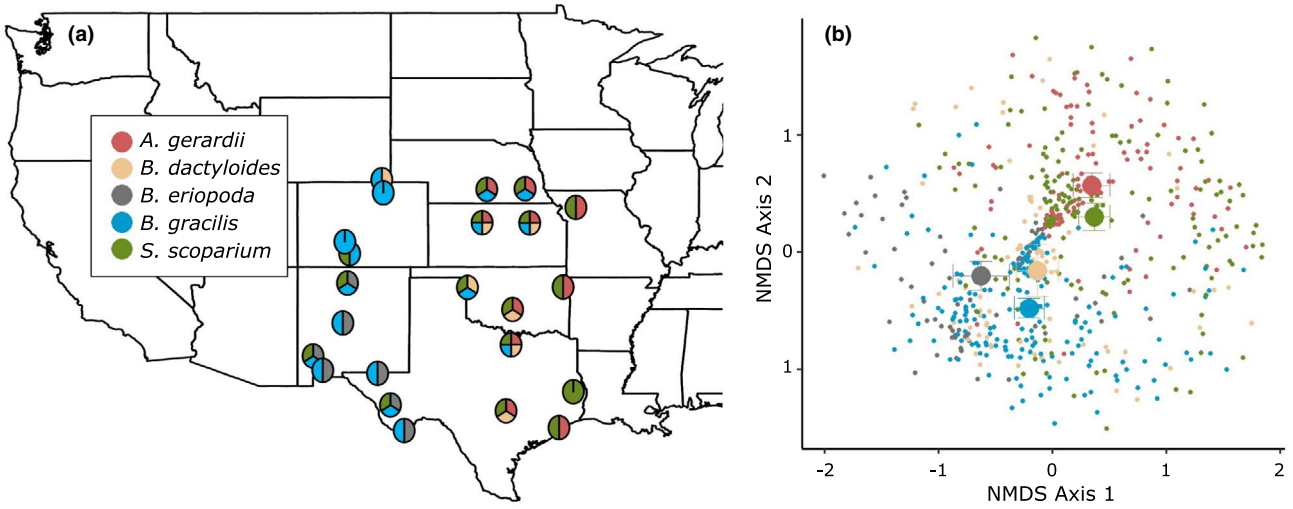

FIGURE 1 (a) Map of sampling locations across the North American plains indicating the species sampled at each site by colour in a pie chart. Geographic coordinates and further site details are provided in Supplementary Material, Table S1. (b) NMDS plots showing variation in the composition of root-associated fungi for five grass species: Species pseudo-$F = 4.0$, $p < 0.0001$, 2-dimensional stress = 0.26 due to strong differences in fungal composition among species. Symbols show the NMDS centroid ± bidirectional SE. Samples were pooled within sites, then SE. calculated across sites for each plant species

indicate investment in plant tissue longevity (Reich, 2014). For SLA, we rehydrated field-collected leaves stored in a plant press by placing them in sealed Petri plates with ~100 ml of DI water and incubating at room temperature for 48 h. Hydrated leaves were scanned for leaf area (cm^2) (WinFOLIA, Regent Instruments Inc.), oven-dried at 65°C for 48 h, then weighed. SLA was the rehydrated leaf area divided by leaf mass (cm^2/g). Fine roots (*ca.* 10 segments) were submerged in DI water in a clear plastic tray, and total root length was determined (WinRHIZO, Regent Instruments Inc.). We then oven-dried roots at 65°C for 48 h to calculate SRL: root length divided by mass (cm/g). In the field, we visually estimated folivory for two leaves per plant (Kent et al., 2020).

2.7 | Root microscopy

Roots were prepared using the modified Vierheilig method (Herrera et al., 2010; Vierheilig et al., 1998) for five randomly chosen plants per species × site (details in Appendix S2). Each sample had 125 images optimised for clarity then overlaid with a virtual 10-line reticle using cellSens® software (Olympus). We estimated the proportion of 10 equidistant intercepts that intersected fungal structures (McGonigle et al., 1990), including melanised (dark) septate hyphae, dark septate vesicles, microsclerotia, hyaline hyphae, hyaline vesicles or spores.

2.8 | *Next-generation sequencing*

We extracted total genomic DNA from 20 cm roots using MoBio PowerSoil DNA Isolation Kits (MoBio) and stored DNA at −20°C until PCR amplification. Extracts were quantified spectrophotometrically (ND2000, NanoDrop Technologies) and standardised to 1 ng/μl for PCR. We targeted the fungal Internal Transcribed Spacer 2 (ITS2) of the ribosomal RNA gene (Blaalid et al., 2013; Schoch & Fungal Barcoding Consortium, 2012). We PCR-amplified the ITS2 with primers fITS7 (Ihrmark et al., 2012) and ITS4 (White et al., 1990). Both forward and reverse primers were appended with unique 12bp DNA multiplex identifiers (MIDs) to reduce error due to tag switching (Carlsen et al., 2012). Primers were trimmed with trim.seqs; additional methods are provided in Appendix S3. Raw paired-end sequence data are available via Sequence Read Archive (SRA) at NCBI (National Center for Biotechnology Information) under BioProject PRJNA705365; BioSamples SAMN18083331-SAMN18084011.

2.9 | Bioinformatics

We processed sequence data using mothur (1.38.1, Schloss et al., 2009). Sequences in paired-end.fastq files were contiged into 48,000,648 total reads. We removed sequences with ambiguous bases, mismatches to MIDs, more than one mismatch to primers, or homopolymers longer than 8 bp. The 30,408,658 remaining reads were truncated to the length of the shortest high-quality reads (236 bp) to facilitate pre-clustering of near-identical reads (Huse et al., 2008). We further screened remaining sequences to remove putative chimeras (UCHIME) (Edgar et al., 2011). We clustered data into OTUs using VSEARCH (Rognes et al., 2016) at 97% sequence similarity and assigned taxonomy using Naïve Bayesian Classifier (Wang et al., 2007) with UNITE INSD as the reference (Köljalg et al., 2013). We removed reads without Kingdom-level assignments. The final dataset had 20,716,515 reads, with mean = 30,376 ± 26,066

SD reads per sample. We rarefied data to 10,000 reads per individual plant to avoid biases from sequence yields (Gihring et al., 2012). To reduce potential sequencing errors, we omitted rare OTUs with sequence counts ≤10 (Brown et al., 2015; Nguyen et al., 2015; Oliver et al., 2015). In mothur, we iteratively calculated Good's coverage (ratio of local OTU singletons to total number of sequences per sample) which averaged 0.994 ± 0.003 SD across all samples. We then calculated OTU richness (S_{obs}), the complement of Simpson's diversity (1-*D*: 1- $\sum p_i^2$) and Simpson's evenness (*E*: 1-$\sum p_i^2/S_{obs}$), where p_i is OTU frequency per sample.

2.10 | Analysis (1) Does the diversity, composition or abundance of root-associated fungi vary with latitude and are latitudinal gradients similar across host plant species?

General linear mixed-effects models tested whether plant species differed in latitudinal gradients of fungal diversity, richness, evenness or root colonisation (fungal abundance in host tissue). Fungal metrics were predicted by latitude, plant species identity and the species × latitude interaction. Models included the random effects of east-west gradient identity and site nested within gradient (Table S1) using the *lmer* function in <lme4> (Bates et al., 2015; R Core Team, 2020). Significant grass species ×latitude interactions were decomposed via secondary analysis for each grass species. The model for *B. eriopoda*, which occurred only on the western gradient, lacked the gradient random effect. We chose the best linear or quadratic model for latitude using the second-order Akaike's Information Criterion (*AICc*). Analyses met assumptions of normality of residuals and homogeneity of variances following square-root transformation of evenness and log-transformation of root colonisation. Data curation, analysis and graphics scripts are available through the Environmental Data Initiative (Rudgers et al., 2021).

We tested for latitudinal gradients in fungal composition using perMANOVA in Primer v. 6.1.10 on the Bray-Curtis distance from the matrix of rarefied proportional abundances of each fungal OTU (Clarke & Gorley, 2015). We binned sites into categories (North, North-Central, South-Central, or South, Table S1) to overcome the perMANOVA restriction to categorical variables. Models included latitude, grass species identity, species × latitude and the random effects of east-west gradient and site nested in gradient. We visualised fungal composition using non-metric multidimensional scaling analysis (NMDS) with 500 random restarts and the Bray-Curtis distance metric (Clarke & Gorley, 2015). Three-dimensional NMDS solutions ($k = 3$) yielded improved stress (<0.2) over 2D solutions. We then ran distance-based linear models (DIST-LM) for each grass species individually using latitude as a continuous rather than categorical variable (Clarke & Gorley, 2015). To explore which fungal taxa contributed to differences among grass species or latitudes, we used indicator species analysis (Dufrêne & Legendre, 1997) in *labDSV* (Roberts, 2018) in combination with SIMPER (Clarke & Gorley, 2015) to obtain the top five indicators per group, following Lagueux et al. (2021). We also regressed the rarified proportional abundance of OTUs against latitude (*lm* function, R Core Team, 2020), and reported the top 10 significant ($p < 0.05$) indicators.

2.11 | Analysis (2) Does the composition of non-mycorrhizal root-associated fungi converge near host range edges?

We used permDISP to examine divergence among individual plants in fungal composition (Clarke & Gorley, 2015) and to test the hypothesis that fungal composition was more homogenous among plants nearing the hosts' latitudinal range edge. Models for each host grass compared dispersion among latitudinal categories (North, North-Central, South-Central or South), each containing similar numbers of samples (Table S1).

2.12 | Analysis (3) What is the relative importance of climate, edaphic factors, or host traits as predictors of root-associated fungal community structure?

We examined environmental correlates of root-associated fungal community structure for each grass species. For fungal composition, we calculated the site average for the rarefied proportional abundance of each fungal OTU to build Generalized Dissimilarity Models (GDM) (e.g. Glassman et al., 2017) that account for spatial non-independence among sites using latitude and longitude coordinates. GDMs examine how species turnover increases with spatial distance among sites, and their *I*-splines describe nonlinear relationships between fungal composition and environmental variables. This approach is particularly important because the same unit increase in, for example, soil moisture may matter more in dry sites than wet sites, producing a nonlinear relationship to fungal composition. Each GDM included 3 climatic variables (annual precipitation, growing degree days and soil gravimetric water content), 3 edaphic variables (soil pH, ammonium and total phosphorus) and 3 plant traits (SLA, SRL and folivory) to give equal weight to each category of predictor. We eliminated multicollinearity by removing correlated predictors, retaining ammonium over nitrate and soil pH over SOM based on initial univariate analyses. All predictors were scaled to mean = 0 and SD = 1. We obtained *p*-values for best models via backward selection in *gdm.varImp* (Ferrier et al., 2007). At three sites, one grass lacked SRL data due to limited root tissue; we interpolated these three missing observations using species-specific regression of SRL on latitude. We reported percentage deviance for GDM model fit following 100 permutations.

For fungal diversity metrics and root colonisation, we used model selection procedures using *AICc* on univariate regressions fit via maximum likelihood to rank the environmental predictors of fungal diversity, evenness, richness, or root colonisation among climatic, edaphic, or host plant traits for each grass species. These general

linear mixed-effects models included the random effects of east-west gradient and site nested within gradient (<lme4>, Bates et al., 2015), and we determined marginal R^2 (Lefcheck, 2016).

3 | RESULTS

3.1 | Community structure of non-mycorrhizal root-associated fungi in plains grasses

A total of 20.7 million high-quality reads representing 7608 OTUs had the following distribution of sequences among grasses: *A. gerardii* (18% of sequences for 120 individuals), *B. dactyloides* (12% for 84 individuals), *B. eriopoda* (13% for 96 individuals), *B. gracilis* (29% for 192 individuals) and *S. scoparium* (28% for 190 individuals). Grasses differed in root-associated fungal taxonomic composition in both sequence-based (Figure 1b; Table 1, $p < 0.0001$) and culture-based datasets (Appendix S1). *B. gracilis* and *B. eriopoda* overlapped the most in fungal composition, and *Andropogon gerardii* and *B. gracilis*/*B. eriopoda* differed the most. Grass species also differed in average root colonisation by non-mycorrhizal fungi (Table 1, $p = 0.0003$). *Bouteloua eriopoda* had the most colonisation (mean ± SE, 56 ± 12%), *S. scoparium* the least (36 ± 5%), and other species were intermediate: *A. gerardii* =38 ± 7%, *B. gracilis* = 44 ± 4% and *B. dactyloides* = 49 ± 6%.

The majority of ITS2 sequences belonged to phylum Ascomycota (70%) with 28% Basidiomycota. Arbuscular mycorrhizal fungi were infrequent (<2% Glomerales), and basal fungi were rare (Chytridiomycota – 0.03%, Rozellomycota – 0.01% and Mucoromycota – 0.51%). Few reads were off-targets (Plantae, Cercozoa – 0.06%; removed from dataset) or unclassified beyond Kingdom Fungi (0.7%). Within the Ascomycota, the most common orders were Pleosporales (28% of total sequences), Hypocreales (11%) and Sordariales (7%). Within Basidiomycota, Agaricales (16%) and Auriculariales (2%) were most common. Among taxa with genus-level assignments, *Fusarium* was abundant (1% of total sequences), as well as *Marasmius* (3%), *Periconia* (2%) and *Moniliophthora* (2%). In culture, Ascomycota comprised 96% of Sanger-sequenced isolates, and Basidiomycota 4% (Appendix S1). Although we cultured and Sanger-sequenced 1,033 fungal isolates, many taxa lacked generic assignments: unclassified Pleosporales represented 18% of all isolates we sequenced, unclassified Ascomycota were ~5% and unclassified Nectriaceae were 4%.

Of the 25 indicator taxa most responsible for differences among grass species, 80% were Ascomycota and 12% were Glomerales. Glomerales taxa thus contributed disproportionately to grass-species specificity, given that Glomerales were a low percentage of total reads (<2%) because we chose primers that are best for non-mycorrhizal taxa. *Andropogon gerardii* was best identified by two *Fusarium* species complexes (order Hypocreales) (Table S2). *Bouteloua dactyloides* indicators included two poorly classified Glomerales and *Gaeumannomyces incrustans* (Magnaporthales), a turfgrass pathogen (Landschoot & Jackson, 1989). Indicators for *B. eriopoda* were two Sordariales (incl. *Chaetomium*), *Curvularia spicifera* (Pleosporales) and an unclassified Marasmiaceae (Basidiomycota). All indicator taxa for the two most widespread grass species, *B. gracilis* and *S. scoparium*, were Ascomycota. Three of the five top indicators of *B. gracilis* were Pleosporales, including two *Darksidea* spp. Indicators for *S. scoparium* spanned four orders of Ascomycota; resolved taxa were putative saprobes: *Xylaria* sp. (Xylariales) and *Biappendiculispora japonica* (Pleosporales) (Tanaka & Harada, 2003). We successfully cultured 56% of the host-plant indicator taxa that were identified by IT2 environmental sequences (Appendix S1).

(1) Does the diversity, composition, or abundance of root-associated fungi vary with latitude, and are latitudinal gradients similar across host plant species?

Latitudinal gradients in fungal diversity and composition differed among grass species (Latitude × Species, Table 1). The expected linear poleward decline in diversity occurred only in *A. gerardii* (Figure 2a, Table 1). In *B. dactyloides*, fungal OTU richness peaked at the highest and lowest latitudes (Figure 2b, Table 1, quadratic), and fungal evenness had a similar latitudinal "U" pattern in *B. gracilis* (Figure 2i). Conversely, evenness peaked at mid-latitudes in both *B. dactyloides* (Figure 2g) and *B. eriopoda* (Figure 2h). Only *B. dactyloides* had a latitudinal trend in root colonisation, with greater colonisation of roots for plants at high latitudes (Figure 2l; Table 1, $p = 0.0853$).

Fungal composition varied with latitude across all grasses combined (Table 1, Latitude $p = 0.0218$, Table S3), although latitude did not explain a large fraction of the variation in fungal communities within individual grass species (Figure 3, $R^2 = 0.03 – 0.09$). The sharpest latitudinal cline in composition occurred in *A. gerardii* (Figure 3a), which also had the strongest latitudinal gradient in richness (Figure 2a). Latitudinal pattern in fungal composition was next strongest for *B. dactyloides* (Figure 3b), then *B. eriopoda* (Figure 3c; Table 1, Table S3). The culture-based dataset revealed similar latitudinal gradients to the amplicon sequences (Appendix S1).

Indicator taxa for latitude were dominated by Ascomycota, which comprised 94% of taxa that increased poleward in their relative abundance (positive β) and 77% of taxa that increased towards the equator (negative β, Table S4). All other grass-specific latitudinal indicators were Basidiomycota (Table S4). Of the latitude indicators, 58% were cultured (Table S4). Pleosporales dominated indicators that increased poleward (44% of total); other taxa included *Mycena adonis* (Agaricales, Basidiomycota), *Lachnum* spp. (Helotiales), and *Gelasinospora saitoi* as well as unresolved Sordariales. Of the indicators that decreased poleward, 30% were Hypocreales (including *Trichoderma gamsii* and *Fusarium chlamydosporum*), 28% were Pleosporales (including *Periconia macrospinosa*, *Paraconiothyrium brasiliense* and *Darksidea alpha*) and two were Agaricales (*Marasmius curreyi* and a *Moniliophthora* sp.).

TABLE 1 Statistical results of mixed-effects models examining the effects of latitude on the root-associated fungi of grass species together as well as each of the five grass species tested individually. Analyses of composition (perMANOVA, *pseudo-F*) and dispersion (permDISP) used latitude as four categorical bins. For individual grass species, columns indicate the fungal response variable examined, providing test statistics for the slope (β) or quadratic term (γ) for latitude, if significant, the standard error (SE) of these parameter estimates, and the *P*-value for latitude as a continuous variable. **p*-values <0.05 are shown in bold, and ***p* < 0.1 in italics, ****p* < 0.001

	OTU richness		(Simpson Evenness)$^{0.5}$		Composition		Dispersion		Colonisation	
All grass species	χ^2	*p*	χ^2	*p*	*F*	*p*	*F*	*p*	χ^2	*p*
Species	10.28	**0.0359**	31.44	**<0.0001**	3.98	**0.0001**	5.33	**0.0024**	13.19	**0.0104**
Latitude	0.10	0.7495	2.72	*0.0994*	1.91	**0.0218**	65.61	**0.0001**	0.04	0.8368
Latitude^2	0.11	0.7377	2.79	*0.0951*					0.04	0.8335
Latitude × Species	14.12	**0.0069**	24.90	**<0.0001**	1.68	**0.0001**	39.48	**0.0001**	9.49	*0.0501*
Latitude^2 × Species	15.61	**0.0036**	25.03	**<0.0001**					9.71	**0.0456**

	Est.	SE	*p*	Est.	SE	*p*	Est.	SE	*p*	*F*	*p*	*F*	*p*	Est.	SE	χ^2	*p*
A. gerardii	−19.53	6.88	**0.0045**	−9.62	3.71	**0.0094**	0.0002	0.0015	0.9029	1.60	*0.0850*	206.82	**0.0001**	0.78	0.72	0.38	0.5396
B. dactyloides	−398.56	47.66	**0.0001**	−258.26	28.69	**<0.0001**	0.1204	0.0266	**<0.0001**	0.91	0.6200	34.33	**0.0001**			2.96	*0.0853*
(γ)	5.43	0.69	**0.0001**	3.59	0.40	**<0.0001**	−0.0016	0.0004	**<0.0001**								
B. eriopoda	17.45	18.32	0.3409	10.55	9.72	0.2779	0.2485	0.0687	**0.0003**	0.93	0.5269	35.49	**0.0001**	−0.02	0.07	0.06	0.7932
(γ)							−0.0039	0.0010	**0.0002**								
B. gracilis	0.34	6.37	0.9582	1.83	3.53	0.6048	−0.0898	0.0338	**0.0078**	1.56	**0.0196**	28.07	**0.0001**	−0.02	0.05	0.11	0.7320
(γ)							0.0013	0.0005	**0.0069**								
S. scoparium	−8.22	7.39	0.2655	−4.27	4.03	0.2891	−0.0020	0.0014	0.1531	1.47	*0.0877*	13.00	**0.0001**	−0.01	0.07	0.01	0.9044

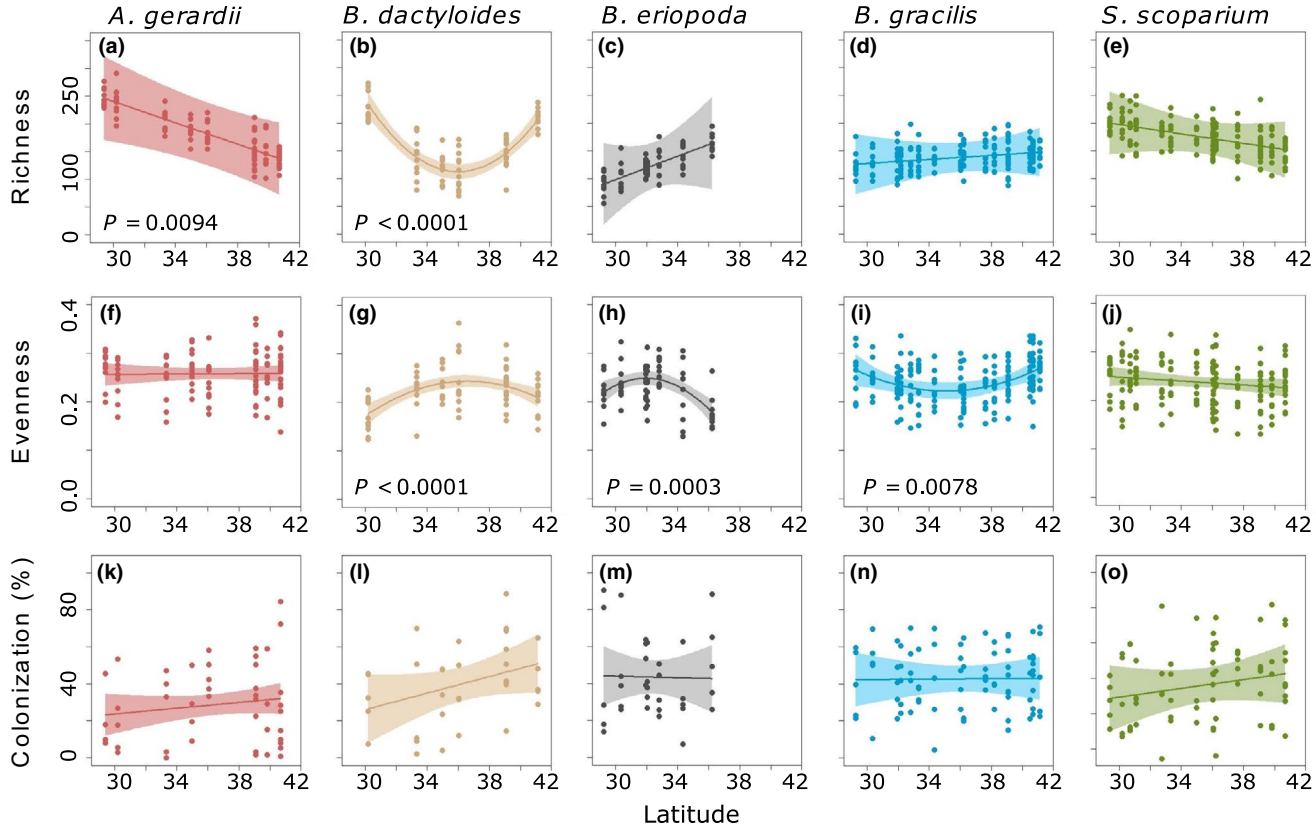

FIGURE 2 Relationships between fungal OTU richness (a-e), evenness, as square-root of the inverse Simpson evenness, *E* (f-j), or root colonisation (%, k-o) against latitude for each of five grass species: *Andropogon gerardii*, *Bouteloua dactyloides*, *B. eriopoda*, *B. gracilis* and *Schizachyrium scoparium*. *p* values for significant ($p < 0.05$) trends in latitude are from a mixed effects general linear model for each grass species. Full statistical results are in Table 1. Samples were pooled within sites, then SE calculated across sites

(2) Does the composition of non-mycorrhizal root-associated fungi converge near host range edges?

All host grass species had significant latitudinal gradients in dispersion, or turn-over, in fungal composition (Table 1, $p < 0.0001$). Dispersion was smallest at sites near grass species' range edges (Figure 3, Figure S1, Table S3). In the desert grass *B. eriopoda*, dispersion decreased northward towards its northern range edge (Figure 3c), whereas other species were sampled approaching their southern range limits, where fungal communities became more similar among host individuals. Among the grasses, *S. scoparium* had the greatest dispersion across all sites and samples (Figure 3e, Table 1, Species $p = 0.0024$), even though *B. gracilis* was sampled at the most sites (Figure 1a, Table S1).

(3) What is the relative importance of climate, edaphic factors, or host traits as predictors of root-associated fungal community structure?

Grass species differed in the relative importance of environmental predictors of root fungal composition (Figure 4). A complex set of climatic, edaphic and plant trait variables explained 17% of variability in fungal composition across grass species together (Figure 4a, $p = 0.02$) and 9–65% of variation in composition for individual grass species (Figure 4b-f). For grasses together, the strongest single predictor was soil pH (Figure 4a). However, individual grass species differed in the rank importance of environmental predictors of fungal composition. Soil pH was the best predictor of composition for *B. dactyloides*, *B. eriopoda* and *B. gracilis*, although the best model for *B. eriopoda* was non-significant (Figure 4d), and in every species, fungal composition was most sensitive to soil pH at values that occurred below the mean pH (nonlinearity). Specific leaf area outranked edaphic factors for predicting fungal composition in *A. gerardii* (Figure 4b). In contrast, soil phosphorus and, surprisingly, folivory were the best predictors of composition in *S. scoparium* (Figure 4f). The degree of nonlinearity in the *I*-splines was greatest for plant trait-related variables, indicating that the sensitivity of fungal composition varied with average values of these traits.

Like fungal composition, root colonisation was also best explained by different environmental predictors for each grass species (Figure 5). No models explained significant variation in non-mycorrhizal root colonisation of *A. gerardii* (edaphic $R^2 = 0.06$, traits $R^2 = 0.03$, climate $R^2 = 0.02$, Figure 5a). In contrast, edaphic factors

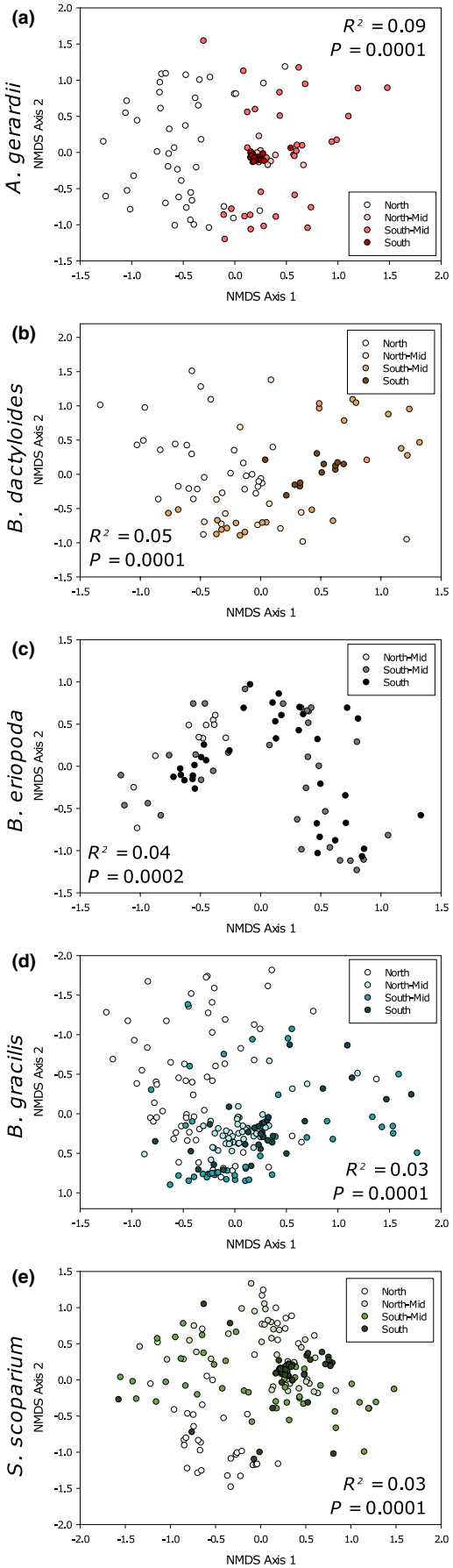

FIGURE 3 Latitudinal patterns in fungal composition shown by NMDS plots for next-generation sequencing of the ITS2 region from roots of five grass species: *Andropogon gerardii* (a), *Bouteloua dactyloides* (b), *B. eriopoda* (c), *B. gracilis* (d) and *Schizachyrium scoparium* (e). Stress of these ordinations ranged from 0.16 to 0.19. Points are coloured by sample latitudinal affiliation, where white is north (N), light shade is north-middle (NM), medium shade is south-middle (SM) and dark shade is south (S). Trends are shown by *p* values for the influence of latitude from DIST-LM for each grass species. Note the tight clustering of samples collected at range edges (N for *B. eriopoda*, or S for other plant species). Table 1 has additional results

explained 24% of variation in root colonisation of *B. eriopoda* and 23% for *B. gracilis*. Soil pH was the most common edaphic predictor of root colonisation and was statistically significant for three grass species (Figure 5b,d,f). For *B. gracilis*, which spanned a wide range of soil pH, the relationship was hump-shaped, with a peak near pH 7.0. For *B. dactyloides*, which occupied a narrow range of soil pH, root colonisation declined in more basic soils (Figure 5b). In contrast, for *B. eriopoda*, which occurred in soils with relatively high pH, characteristic of desert grasslands with calcic soil deposits, root colonisation increased linearly with soil pH up to the sampled maximum of 8.4 (Figure 5d). Soil phosphorus was an additional predictor of root colonisation for *B. gracilis* (Figure 5g), as was specific root length for *B. eriopoda* (Figure 5e). In contrast, climate variables explained the most variation in root colonisation for both *B. dactyloides* (22%) and *S. scoparium* (13%). However, whereas precipitation was the strongest climate predictor of root colonisation for *B. dactyloides* (Figure 5c), growing degree days ranked best for *S. scoparium* (Figure 5i).

4 | DISCUSSION

Our survey of foundation grasses of North American plains demonstrated that most metrics of root-associated fungal diversity lacked the poleward declines that occur commonly in plants, animals, and even in some studies of soil fungi (e.g. Tedersoo et al., 2014). A linear poleward decline in fungal diversity occurred in only one of five grasses (*A. gerardii*, big bluestem). The absence of a latitudinal diversity gradient was also supported by the result that climatic variables, which often underlie latitudinal gradients in biodiversity (Hawkins et al., 2003; Pianka, 1966), did not strongly structure root-associated fungal communities relative to other environmental variables. This result is in line with a similar study that focused instead on ectomycorrhizal root fungi in the arctic. Specifically, species richness of ectomycorrhizal fungi on willow and *Dryas* plants did not vary with latitude despite geographic structure to overall fungal community composition; although in contrast to our results, host plant identity did not structure fungal community composition (Timling et al., 2012). However, our result contrasts against a recent global meta-study of >3,000 soil samples, in which climate variables accounted for ~65% of explained variation in soil fungal communities

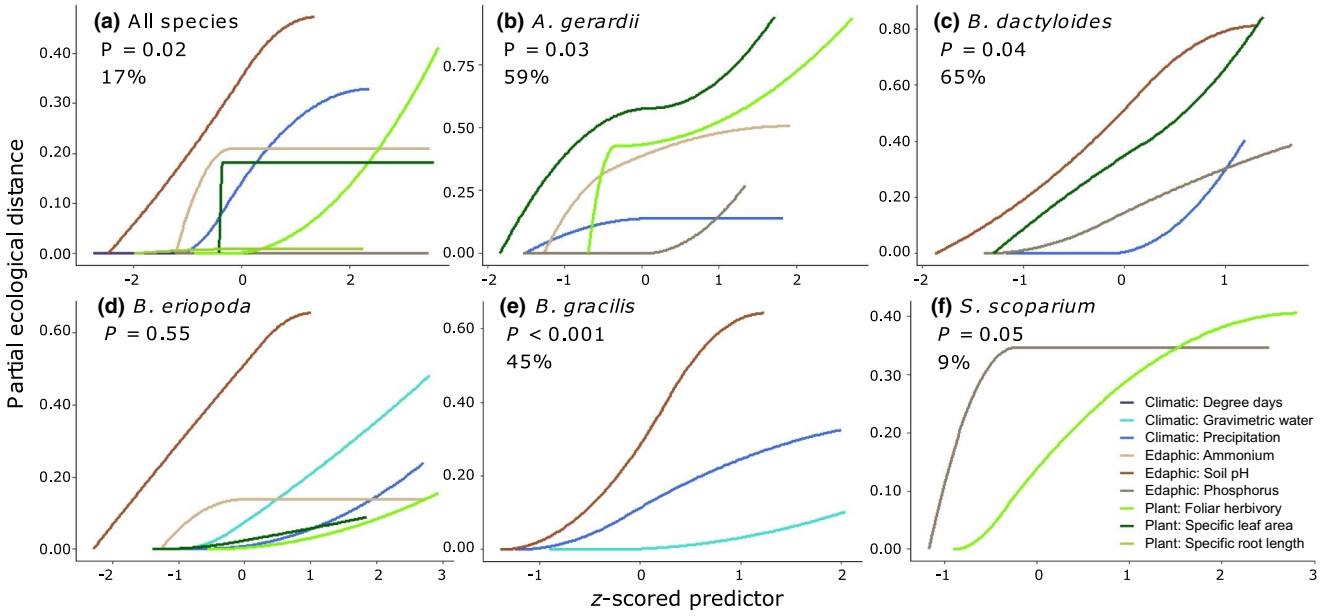

FIGURE 4 *I*-splines from the best model among generalised dissimilarity models (GDM) comparing climatic factors (blue colours), edaphic factors (brown colours), or plant traits (green colours) as predictors of root-associated fungal composition. The proportion of deviance explained by each best model is given in the upper left corner of each plot for statistically significant models. All species combined are shown in (a), followed by *Andropogon gerardii* (b), *Bouteloua dactyloides* (c), *B. eriopoda* (d), *B. gracilis* (e) and *Schizachyrium scoparium* (f)

(Vetrovsky et al., 2019). The relatively low explanatory power of latitude for root fungi was comparable to several prior results for soil fungi (e.g. Vetrovsky et al., 2019, but see Arnold & Lutzoni, 2007 for leaf fungi). Perhaps root-associated fungi are more buffered from climate variables than are foliar fungi or fungi that reside primarily in soils rather than inside roots. In addition, the apparently wide ecological plasticity of root-associated fungi (Challacombe et al., 2019; Egidi et al., 2019; Porras-Alfaro & Bayman, 2011) may explain why, in contrast to many other organisms, these fungi were less sensitive to climate than to edaphic or host-associated variables. Alternatively, the lack of a classic latitudinal diversity gradient could occur because our gradient did not extend to the tropics as we constrained sampling to coincide with host plant distributions. However, the grass species that had a significant latitudinal decline in fungal diversity was not the species with the widest range of latitudes sampled, suggesting a wider gradient would not change the biogeographic patterns detected within individual plant species. Further, our results demonstrate, as others have suggested (Brunel et al., 2020; Lagueux et al., 2021; Schroeder et al., 2019), that accounting for host species identity can help disentangle biogeographic patterns of symbionts from those of their host species. Ultimately, both global studies and host-constrained, regional studies will be needed to advance current knowledge. In addition to rejecting the hypothesis of latitudinal diversity declines for root-associated fungi, our work revealed intriguing latitudinal clines for some common, non-mycorrhizal fungal taxa. Most notable were poleward increases in the relative abundance of several Pleosporales taxa and poleward declines in Hypocreales. Common root-associated taxa that decreased in relative abundance poleward included species in the genera *Darksidea*, *Fusarium*, *Periconia*, *Paraconiothyrium* and *Trichoderma*.

Host species identity strongly structured relationships between the environment and fungal composition or colonisation of roots (Figures 4 and 5). Host plant identity was the most important source of biogeographic variation in fungal composition (Figure 1b, Table 1—*p*-values for plant species identity outranked those for latitude across all response variables but dispersion). Such large effects of plant identity or plant phylogenetic relatedness on plant-associated microbes have been reported previously (e.g. Kazenel et al., 2019; Sweeney et al., 2020; Yang et al., 2019). Yet, other studies have alternatively suggested that edaphic factors override the influence of host plant identity on microbiome composition (Erlandson et al., 2018; Glynou et al., 2016). Our results emphasise that geographic variation in the composition of plant microbiomes cannot be readily generalised across host species, even for plants within the same clade (e.g. Poaceae), and that increasing plant taxonomic resolution in efforts to globally scale the biogeography of plant-microbe symbioses (e.g. Steidinger et al., 2020) could produce much more complex results than current studies, which typically ignore or lump across plant species identities. Recent reviews and meta-analysis demonstrated that most prior studies have not studied multiple host plant species across the same geographic gradients in order to detect the degree of host-specificity in environmental correlates of microbiomes (Brunel et al., 2020; Kivlin et al., 2017). Thus, improved study designs could determine whether host-specificity in environmental covariates of microbiomes, as we have demonstrated here, is also a general phenomenon.

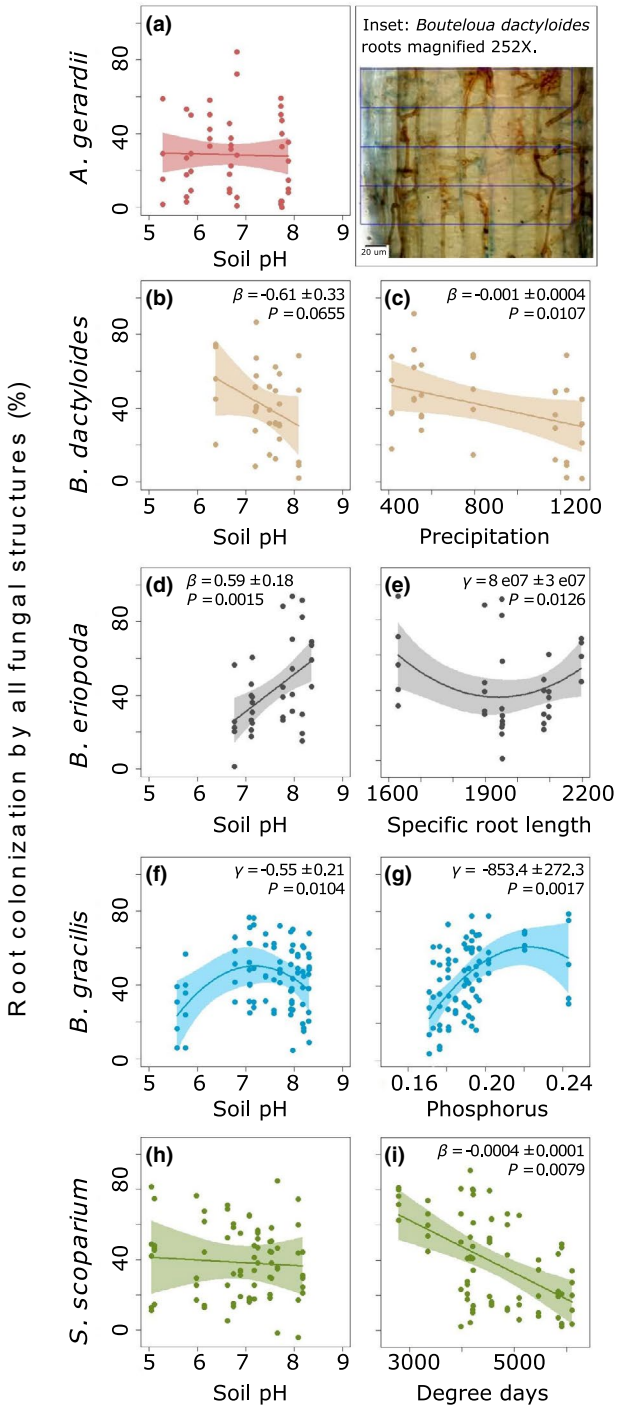

FIGURE 5 Best climatic, edaphic, or plant trait predictors of root colonisation by non-arbuscular mycorrhizal fungi for *Andropogon gerardii* (a, non-significant), *Bouteloua dactyloides* (b-c), *B. eriopoda* (d-e), *B. gracilis* (f-g) and *Schizachyrium scoparium* (h-i). *Inset*: Image of colonised roots with grid overlay at 252× magnification

A complex set of edaphic, plant trait and climate variables predicted root-associated fungal composition within host plant species. Among these, edaphic characteristics, particularly soil pH, were often, but not always, the best environmental correlates of fungal composition. This result corroborates prior work highlighting soil pH as a regional or global driver of *soil* microbial communities (Fierer & Jackson, 2006; Lauber et al., 2009; Tedersoo et al., 2014, 2020). However, our study newly suggests an influence of foliar herbivory and plant-specific leaf area (Figures 4 and 5). Why might these traits be important to root fungi? We posit that the carbon costs of herbivory and leaf construction and maintenance constrain the quantity or quality of plant root exudates (Sasse et al., 2018) or other resources that influence fungal composition belowground. In addition, our work advances understanding because we sought and detected nonlinear relationships between fungal communities and environmental covariates, which help to refine predictions on potential responses to environmental change (Rudgers et al., 2020).

Despite the absence of typical latitudinal gradients, in all grasses, root fungi converged in composition as sampling approached one edge of the host plant's latitudinal range. This homogenisation was not caused by simple north-south gradients in fungal communities because *B. eriopoda* was sampled to its northern, rather than southern, range edge, yet had a similar pattern of convergence. Biotic or abiotic stressors at host range edges could constrain the degree of individual differentiation among plants in microbiome composition that results from stochastic processes, as has been detected by stress experiments in other community types (e.g. Chase, 2007). Alternatively, low plant genetic diversity at range edges could constrain fungal diversity and dissimilarity among individual plants. For example, Gao et al. (2020) reported that drought stress in sorghum plants increased the stochasticity of community assembly for belowground microbes and decreased selection, likely due to the smaller total community size under stress. We are unaware of prior studies on the beta-diversity of plant microbiomes near host range edges. Work investigating species interactions in setting range limits (Louthan et al., 2015) may benefit from greater attention to host-microbe interactions (e.g. Lynn et al., 2019).

Environmental variables explained 9%–65% of variation in fungal composition and 6%–24% of variation in root colonisation across foundation grass species, suggesting the possibility of unmeasured covariates, particularly for *S. scoparium*. However, this magnitude of explanatory power was similar to other regional or global fungal surveys that examined environmental correlates and plant or vegetation characteristics (e.g. Egidi et al., 2019; Chen et al., 2017; Sweeney et al., 2020, Tedersoo et al., 2002; Vetrovsky et al., 2019). Other factors for future study may increase explanatory power by extending predictors to include fire, grazing history, or other disturbances (Carson et al., 2019; Yang et al., 2020). In addition, past work suggested that fungal composition varies with plant age or life history stage (Chung et al., 2018; Edwards et al., 2018), and a recent study indicated that paired analysis of fungi and bacteria can improve explanatory power due to coupled microbial interactions (Bergelson et al., 2019).

In conclusion, our latitudinal survey of root-associated fungi in foundation grasses across North American plains grasslands demonstrated the primacy of host plant identity in shaping the community structure and abundance of non-mycorrhizal root fungi. Fungi associated with grass roots did not follow typical latitudinal diversity gradients with poleward declines. Additionally, near the edges of

host species' latitudinal ranges, individual plants had the greatest similarity in fungal composition, perhaps indicating a homogenising effect of stressors at range margins. Taken together, our results suggest that non-mycorrhizal fungi in grass roots will be more sensitive to future changes in host ranges or edaphic factors than to direct effects of climate change, and that these sensitivities will be strongly shaped by the identity of their host plant.

ACKNOWLEDGEMENTS

Many thanks to Sean Morris for assistance with DNA extractions, to Kendall Beals and Jennifer Bell for assistance with field collections, to Katy Beaven for soil analyses, and to Josh Lynn for plant traits assistance. Thanks to Terri Billingsley Tobias, Cedic Ndinga Muniania, Paris Hamm, Terry Torres-Cruz, Adeyemi A. Olanrewaju for sample processing, culture isolation, and sequencing, to Sean Morris and Alina Akhunova for preparing sequence libraries, and to Nora Mitchell for assistance with species distribution maps. Thanks to editor Vincent Merckx and two anonymous reviewers for suggestions that improved the manuscript. All data have been lawfully acquired in accordance with The Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from Their Utilization to the Convention on Biological Diversity, with approved collecting permits for all sites. This work was supported by NSF-DEB#1457309 to AJ, #1619935 to JH, #1457002 to AP, and #1456955 and LTER#1655499 to JR as well as by NSF-EPSCOR#1656006. Material is based upon work supported by the National Science Foundation. Any opinion, findings, and conclusions or recommendations are those of the authors and do not necessarily reflect the views of the National Science Foundation.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

Datasets and R scripts are publicly available as data packages through the LTER network EnvironmentalDataInitiative.org doi through Rudgers, J.A., S. Fox, A. Porras-Alfaro, J. Herrera, D. Kent, L. Souza, Y.A. Chung, and A. Jumpponen. 2021. Biogeography of root fungi in grasslands ver 1. Environmental Data Initiative. https://doi.org/10.6073/pasta/c8255a205f34e101c41063f4cfee850d and through the Sequence Read Archive (SRA) at NCBI (National Center for Biotechnology Information) under BioProject PRJNA705365; BioSamples SAMN18083331-SAMN18084011.

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BIOSKETCH

Jennifer A. Rudgers: Ecological theory has traditionally held that abiotic factors and antagonistic interactions are the primary determinants of population and community dynamics. Mutualisms have received much less attention. Rudgers' research program (rudgerswhitneylab.weebly.com) uses plants, microbes and arthropods to explore how mutualistic interactions affect population dynamics, community structure, ecosystem processes and evolution of species traits. Mutualistic microbes, in particular, contribute important, but often overlooked, diversity in ecosystems. This research team has been collaborating since 2015 to expand understanding of the ecology of plant-associated fungi to increase the realism of experiments and theory and improve predictions on the community-level consequences of environmental change. Rudgers also directs the Sevilleta Long-Term Ecological Research Program (sevlter.unm.edu), funded by the National Science Foundation and featured in this biogeographic survey.

Author Contributions: JR, AP, AJ and JH planned and designed the research. AC, LS, DK, JH, AP and CR conducted fieldwork and lab work. JR, SF and AJ analysed the data. JR and SF wrote the manuscript, and all authors contributed substantial revisions and edits. Thanks to Nora Mitchell for assistance with mapping graphics, and to Editor Merckx and two anonymous reviewers for improvements to the manuscript.

SUPPORTING INFORMATION

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How to cite this article: Rudgers, J. A., Fox, S., Porras-Alfaro, A., Herrera, J., Reazin, C., Kent, D. R., Souza, L., Chung, Y. A., & Jumpponen, A. (2022). Biogeography of root-associated fungi in foundation grasses of North American plains. *Journal of Biogeography*, *49*, 22–37. https://doi.org/10.1111/jbi.14260