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RESEARCH ARTICLE

Tree-fungal interactions across climatic gradients: What is the potential for tree niche expansion via varying fungal associations?

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Abstract

1. Tree species persist across wide climatic gradients, potentially facilitated by varying species interactions. We investigated how tree species' associations with fungal communities varied with climatic gradients across their range.
2. We partnered with volunteers to sample tree roots across the eastern United States, with >1000 participants providing samples from 20 states. We characterized fungal communities via amplicon sequencing and tested how fungal guilds and individual taxa varied across climate gradients, as well as how fungal guilds and taxa correlated with seedling performance in a field experiment representing four climate conditions.
3. Relative abundance of non-mycorrhizal endophytes (NME) increased in hotter and drier locations, guild-wide and for individual taxa. Relative abundance of ectomycorrhizal (EM) fungi declined in drier sites, while that of many individual EM fungi increased with colder temperatures. Seedling performance increased with greater relative abundance of arbuscular mycorrhizal (AM) fungi and intermediate abundance of NME in hot, dry conditions and individual NME taxa had on average positive correlations with seedling growth in these conditions. One of the NME, *Cladosporium*, emerged as a candidate taxon for niche expansion in *Acer* trees due to its increased abundance and positive association with seedling growth in hot, dry conditions in field conditions.
4. *Synthesis*. Climatic niche breadth may reflect both intrinsic tree traits and varying relationships with microbes. NME represents an intriguing group whose impact on current and future tree ranges deserves further investigation.

KEYWORDS
arbuscular mycorrhizal fungi, biogeography, ectomycorrhizal fungi, endophyte, fungal ecology, mycorrhizae, plant-soil (below-ground) interactions, temperate forest, tree

1 | INTRODUCTION

Many tree species span wide geographic ranges, occurring across locations with widely different climatic conditions. How single tree species can tolerate such a wide range of conditions remains unclear. Moreover, as climates change rapidly, will these tolerance ranges stay fixed for tree species or could trees expand their observed climate tolerance in the face of increasingly stressful conditions? Three categories of mechanisms could explain the broad environmental tolerance of trees. First, trees are modular and plastic, allowing individuals to respond to a wide range of conditions through phenotypic plasticity (Ackerly et al., 2000; Agrawal, 2001; Gratani, 2014; Valladares et al., 2006). Second, tree species are not genetically uniform, and tree populations can become adapted to their local conditions given sufficient genetic variability within populations and low enough gene flow between populations (Kawecki, 2008; Savolainen et al., 2007; Wu & Ying, 2004). Finally, plant phenotypes can be shaped by interactions with other species. Trees interact with a wide range of microbes in their roots that can affect their tolerance to both climatic and soil conditions (Kipfer et al., 2012; Kivlin et al., 2013; Lehto & Zwiazek, 2011; Ware et al., 2021). Could trees expand their climate niche breadth by associating with different microbial taxa across their species range?

Predictions of future forest composition rely heavily on the assumption that the current geographic range of a tree species represents the extent of the climatic conditions that species can tolerate. Future ranges are predicted by mapping where the tree species' currently inhabited climate will occur geographically in the future (Iverson et al., 2019; Thuiller et al., 2008). However, if the climate niche of a tree species is not fixed, this approach may under- or overestimate the extent and location of future ranges. For instance, naturalized plant species regularly occupy climates in their introduced region far outside of the 'climate envelope' occupied in their native range (Early & Sax, 2014). Similarly, models based on spatial climate variation perform worse than those based on temporal climate variation when predicting tree demographic responses to recent climate change (Perret et al., 2024).

Since trees have long generation times, evolutionary changes in climate niches are often assumed to be slow relative to current rates of climate change (Etterson & Shaw, 2001). However, climate niche expansion via alteration of microbial associates could occur rapidly and may represent an underappreciated source of stability in tree range dynamics (Lankau et al., 2015). Moreover, if the current range boundaries of tree species already represent the hidden action of geographically varying microbial associations, then predictions of future range dynamics in response to changing climates may be inaccurate. For instance, if key microbial mutualists are not present in newly permissive locations, this could inhibit range expansion, as suggested for ectomycorrhizal (EM) tree species (Van Nuland et al., 2024). As both tree and microbial distributions shift in space and novel interactions are formed, tree populations may gain tolerance to previously intolerable climates (Allsup et al., 2023; Allsup & Lankau, 2019).

Plants interact with a wide diversity of microbial groups in their roots, including bacteria, archaea, fungi, protists and nematodes, all of which could affect plant tolerance of climatic conditions in direct and indirect ways. Root-associated fungi have many documented impacts on plant tolerance to climatic conditions (Acuna-Rodriguez et al., 2020; Latef et al., 2016; Marquez et al., 2007; Porter et al., 2020). Mycorrhizal fungi, including arbuscular, ecto- and ericoid mycorrhizal fungi, can affect drought tolerance through increased root surface area leading to higher water uptake and enhanced nutrient acquisition in dry soils (Kipfer et al., 2012; Lehto & Zwiazek, 2011; Ruizlozano et al., 1995). They can also affect soil hydrology by increasing water holding capacity (Lehto & Zwiazek, 2011; RuizLozano & Azcon, 1995; Wilson et al., 2009). Roots are also host to diverse communities of fungi that are not recognized as 'mycorrhizal', in that they do not form typical mycorrhizal structures. This group includes plant pathogens and parasites, but also the 'dark septate endophytes' and other less well studied groups (Mandyam & Jumpponen, 2005). Functionally characterizing taxa in this group is challenging, as their impacts on host plants can vary within and between species and even genetically identical strains based on environmental conditions (Mandyam & Jumpponen, 2015; Marc-André et al., 2018; Schlegel et al., 2016). However, at least some strains have been documented to increase plant drought, heat and cold tolerance through a variety of known and unknown mechanisms (Afkhami et al., 2014; Mandyam & Jumpponen, 2005; Marquez et al., 2007). Of course, detrimental interactions can also influence plant tolerance to abiotic stress; for instance, plant populations at expanding range edges could escape from co-evolved pathogens (McCarthy-Neumann & Ibanez, 2012; van der Putten et al., 2016; van Grunsven et al., 2010); this could allow a range-expanding plant to tolerate more stressful climatic conditions than would be possible in the presence of pathogens. In addition to symbiotic endophytes, roots also harbour looser associations with rhizoplane and rhizosphere microbial taxa, such as saprotrophic fungi, that can influence plant growth via the mineralization of organic nutrients, production of plant hormones and suppression of plant pathogens, among other means (Berendsen et al., 2012; Huang et al., 2014).

Plants can exhibit increased growth in stressful conditions when inoculated with microbial communities that have a prior history with those conditions (Allsup et al., 2023; Allsup & Lankau, 2019; Lau & Lennon, 2012). If microbial community composition varies geographically, then each population of a plant species would interact with a unique suite of microbes (Talbot et al., 2014; Tedersoo et al., 2014; Van Nuland et al., 2023; Větrovský et al., 2019). If these unique microbial communities provide enhanced tolerance to local conditions, then the additive effects could result in a broader range of climates tolerated across the whole plant species range. Moreover, changes in local (alpha) diversity of microbial associates of individual plants might vary across plant species ranges, with synergistic effects on plant species ranges and plant performance in stressful conditions.

To assess whether geographically varying fungal associations may be acting to expand tree species climatic tolerance, we first need to determine if and how the root fungal communities of trees

vary across climate gradients, within and across tree species. Here, we partnered with volunteers to achieve widespread geographic sampling of tree roots from across the temperate forest biome of the eastern United States. We characterized the fungal communities in and on roots using amplicon sequencing and then evaluated how the relative abundance and diversity of fungal guilds and abundance of individual fungal taxa varied across gradients in temperature and aridity.

Patterns of association across geographic space, on their own, cannot determine whether the fungi are promoting individual tree fitness or population stability at climate extremes versus simply responding to their own environmental preferences with no consequence for their host tree—or even parasitizing stressed host trees at the edge of their climate tolerance. Therefore, to provide context to these patterns with respect to impacts on tree performance, we assessed the association of fungal guilds and individual fungal taxa with tree seedling growth in two field sites, each with a rainfall manipulation, representing four contrasting climate conditions.

We used these datasets to address two research questions:

1.1 | Question 1: How does the composition and diversity of root-associated fungal communities vary across climatic gradients?

If tree populations rely on associating with varying fungal taxa to broaden their environmental niche breadth, then we predict that variation in root-associated fungal community structure will correlate with specific climatic gradients, both between and within tree species. Specifically, we predict that tree populations on the dry, hot and cold extremes of their range will associate with different fungi, including altered proportions of functional guilds as well as individual fungal taxa.

1.2 | Question 2: What aspects of fungal communities correlate with seedling performance under contrasting climate conditions?

We predicted that at least some fungal guilds and taxa that vary across climate conditions and will also be positively associated with seedling growth in the corresponding climate condition. These taxa will be strong candidates for potential niche-expanding factors for their tree hosts.

2 | MATERIALS AND METHODS

2.1 | Geographic sampling of root-associated fungi

To achieve broad geographic sampling, we collaborated with volunteers throughout the eastern United States to collect root tissue from forested areas. Participants were recruited through a variety of means, including youth organizations (4-H, K-12 schools) and volunteer programs (Master Naturalists) and via outreach to county and regional extension agents (see https://forestfungi.russell.wisc.edu). We defined 'forested area' broadly as areas with mature tree cover and an unmown understory. We asked participants to collect fine root material from forested areas with the permission of landowners by digging a hole ~0.5–1 m from the base of an identified tree and selecting first and second order roots. Volunteers collected root samples between August and October, with the constraint that trees should still have green leaves when sampled. Roots were shaken to remove as much loose soil as possible. Sampling occurred over the top 10 cm of soil. Volunteer collectors were instructed to collect the finest roots possible. Participants stored collected roots in plastic bags at refrigerator temperature (~4°C) and shipped them to our laboratory in Madison, WI, within 2 days of collection. Overall, samples were received in our lab within 7 days of field collection, at which point they were frozen until used for DNA extraction. Participants recorded the GPS coordinates of each sampling location, the sampling date and the identity of the targeted tree. Between 2015 and 2022, over 1000 participants shipped a total of ~950 samples from 20 states (Figure 1; Table S1).

Once samples were received at our laboratory, they were immediately frozen at −20°C until molecular processing (3–6 months). Samples were thawed, and a single continuous root fragment was selected for processing—approximately 3 cm of root length and 25 mg fresh weight. We selected the finest possible roots from each sample and avoided any roots that appeared discoloured or showed signs of decay. The root fragment was vigorously washed free of any adhering soil with DI water, then placed in a 2 mL microcentrifuge tube with two metallic beads prior to DNA extraction.

2.2 | Field experiment

To investigate how fungal taxa are associated with tree seedling growth in contrasting environments, we used a separate dataset derived from experimentally planted tree seedlings at two locations—in northern Wisconsin (Kemp Natural Resources Station) and central Illinois (Allerton Park and Recreation Center). The Wisconsin site is about 725 km north of the Illinois site. The mean annual temperature (MAT) of the Illinois site ranged from 10.7 to 11.1°C for the 5 years that our seedlings were presented, while the Wisconsin site (MAT) ranged from 3.5 to 5.6°C. For summer aridity, the Aridity Index (AI) of the Illinois site ranged from 0.55 to 0.81, while the Wisconsin site ranged from 0.52 to 0.75. The AI was calculated as the ratio of precipitation to potential evapotranspiration, so lower values indicate drier conditions (see methods below).

This data include a mixture of previously reported data from Allsup et al. (2023), along with additional unpublished data. At each location, seedlings were planted into paired plots, with each pair including a reduced rainfall treatment and a matched ambient rainfall treatment created using plastic shelters that did or did not intercept rainfall (see Allsup et al., 2023 for details). Seedlings were planted

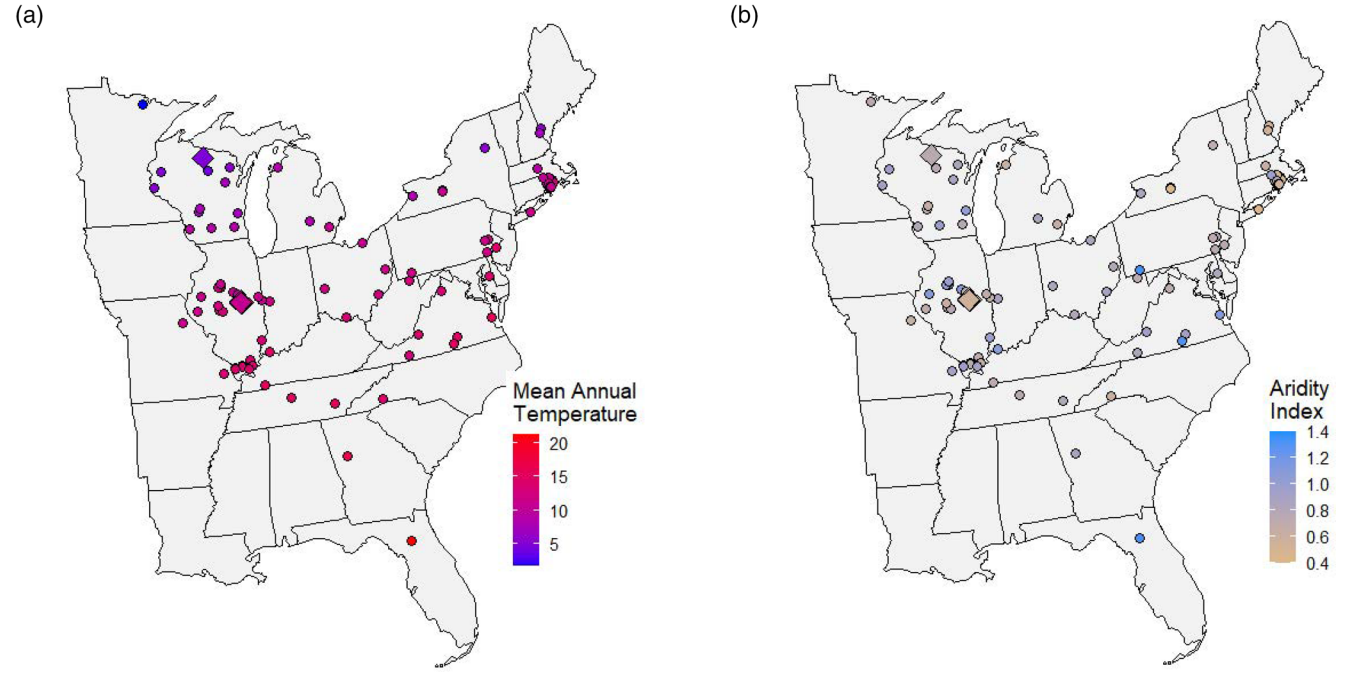

FIGURE 1 Volunteer root sample locations with (a) mean annual temperature and (b) Aridity Index (lower values indicates more arid conditions).

in three cohorts—in 2018, 2019 and 2020—and allowed to grow for 3 years prior to harvesting. Seedlings were germinated in sterilized potting media, then grown in 0.6 L Cone-tainers filled with live soil collected from one of 12 forested sites in Illinois or Wisconsin for 8 weeks prior to planting (Allsup et al., 2023). After three growing seasons, we measured any seedlings still living for stem height and basal diameter and excavated root systems in the late fall. We collected fine root tissue from each harvested seedling and characterized fungal communities using the methods described below. Note that this dataset is censored, as we only have stem diameter and height data on seedlings that survived all three study years. Most planted seedlings died prior to this point, and survival depended on the initial microbial inoculation (Allsup et al., 2023); thus, we lack data on how fungal taxa impacted mortality.

From the field experiment, we included a total of 470 unique seedling samples that had sufficient sequencing information and data on final seedling height and stem diameter. These seedlings came from three planting cohorts (planted in 2018, 2019, 2020). The 2018 and 2019 cohorts were the same set of seedlings used for the analysis of root fungal communities in Allsup et al. (2023)—this represented 309/470 samples (~65%). The 2020 cohort has not been included in any previous analysis.

2.3 | Molecular characterization of fungal communities and host taxa

We characterized the fungal communities in and on root tissue using Illumina sequencing of the ITS2 rRNA gene region using previously described methods (Allsup et al., 2023). We also used the resulting ITS2 sequences, along with other methods, to identify the host genus (see Supporting Information for details). Full methods are presented in the Supporting Information. In brief, root tissue was flash frozen in liquid nitrogen and ground to a fine powder; then DNA was extracted using a commercial kit. The ITS2 region was amplified via PCR using the ITS3-KYO2 (Toju et al., 2012) and ITS4 (White et al., 1990) primers. We chose the ITS2 region primers for this study (ITS-KOY and ITS4) due to their published ability to amplify a wide range of fungi, including Mucromycotina and Glomeromycotina (Toju et al., 2012). The PCR product was then used as the template for a second PCR that added Illumina adaptors and sample-specific barcodes. Libraries were sequenced using Illumina Miseq PE 2×300 chemistry across several runs over the 6-year period. Sequences were denoised, clustered into exact amplicon sequence variants and taxonomically identified using the DADA2 programme (Callahan et al., 2016) as implemented in the QIIME2 pipeline.

Fungal species were assigned to one of six guilds based on the Fungal Traits database (Polme et al., 2020): arbuscular mycorrhizal fungi (AMF), EM fungi, non-mycorrhizal endophytes (NME), saprotrophic fungi, 'other' and unassigned—see Supporting Information for details. The assignment of fungi to functional guilds may be error prone or conceptually fraught (Marc-André et al., 2018). Many taxa exhibit multiple lifestyles, including both saprotrophic and endophytic life stages.

Our method cannot reliably separate truly endophytic taxa from superficial inhabitants of the root zone. A trial with surface sterilized roots found differences in fungal composition of 'sterilized' versus 'non-sterilized' roots, but in surprising ways (see Figures S1 and S2). EM fungi were reduced in relative abundance in sterilized compared to unsterilized roots, likely due to loss of biomass from fungal

mantles, while most groups did not change substantially (Figure S2). Thus, we have reasonable confidence that the fungal communities we analysed primarily represent close associates of roots (rhizoplane or endophytes).

2.4 | Environmental data sources

For each sampling site and collection year, we obtained temperature and precipitation data by month for the 5 years prior to sampling from the PRISM database (PRISM Group, 2004). To quantify aridity, we used the AI calculated over the growing season (May–September) prior to sample collection. The AI is the ratio of total precipitation to potential evapotranspiration and measures the predicted ratio of moisture inputs to losses; higher values indicate wetter conditions, while lower values indicate increasing drought stress for plants. We estimated potential evapotranspiration using the Hargreave approximation method with the spei package in R, using latitude and month to predict external radiation (Begueria & Vicente-Serrano, 2017). MAT was measured as the daily mean temperature averaged over the 12 months prior to soil sampling. MAT was highly correlated with the annual minimum and maximum temperature in our dataset ($r = 0.84$ and 0.81, respectively). Unfortunately, we were not able to obtain direct measurements of soil physical and chemical properties of our sampling sites, as we only receive root material from most of our volunteer collectors. However, we used the USDA Web Soil Survey to approximate the soil order of each sampling location based on collector-provided GPS coordinates (Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture, n.d.). Since our goal was to investigate root-associated fungal communities across climate gradients, we used the soil order information in our models to account for coarse soil factors that could confound our interpretation of climate gradients. However, we acknowledge that variation in specific soil physiochemical properties (texture, pH, soil organic matter) also has an important influence on root-associated fungal communities that we cannot address in this study.

2.5 | Statistical analyses

2.5.1 | Data exclusion

We eliminated any samples for which we could not confidently identify the plant root to at least the genus level (191 samples) or which were identified to an herbaceous species (23 samples), which reduced our dataset from 866 to 652. Based on rarefaction curves, we eliminated any samples with fewer than 500 sequence reads assigned to the Kingdom Fungi, which reduced our dataset from 652 to 512 samples (Figure S3). In total, this resulted in a final set of 512 samples from 81 unique sites and 105 unique site-years, which retained samples from 29 woody genera (at least 33 species). We assigned these woody genera as either arbuscular (AM) or EM associated based on Soudzilovskaia et al. (2020)—dually colonized genera (*Populus*, *Betula*) were included in the EM group for analysis. The included and excluded samples had a similar geographic and climatic range. Climatic and geographic means for the 512 retained samples were: MAT = 10.93 ± 0.13°C, AI = 0.77 ± 0.01, Latitude = 40.80 ± 0.12°N, Longitude = −84.38 ± 0.30°W. Mean values were very similar for the 140 samples excluded for insufficient sequences: MAT = 10.61 ± 0.27°C, AI = 0.79 ± 0.02, Latitude = 40.20 ± 0.26°N, Longitude = −84.70 ± 0.54°W.

2.6 | Question 1: How do root-associated fungal communities vary across climatic gradients?

We used linear mixed models to test how the relative abundance of the six fungal guilds varied across climatic gradients. The relative abundance of each guild was determined by summing the sequence reads of each fungal taxa identified to that guild, then dividing by the total sequence count of the sample. Models included the site MAT, AI over the growing season and longitude as quantitative fixed predictors. Tree genus and estimated soil order were included as crossed random effects (intercepts). All models were run separately for AM- and EM-associated tree genera as the compositional nature of amplicon sequencing makes these fungal communities inherently non-comparable. Additionally, we ran models within specific high sample tree genera (*Acer*, *Quercus*, *Carya*) as well as among all other AM tree samples and all other EM tree samples for a total of five sets of models per fungal guild. This analysis aimed to determine if the overall trends occurred within, as well as across, tree mycorrhizal types.

We used similar linear mixed models to analyse fungal diversity (Shannon index) across climate gradients. For these models, we included the total sequencing depth (of sequences identified to fungi) per sample as an additional covariate to control for the potential to observe more taxa with higher sampling depth. We analysed total fungal diversity as well as diversity calculated within each of the six functional guilds.

We used differential abundance analysis based on generalized linear models (GLM) to quantify the association of individual fungal taxa with climate gradients with the DESeq2 and phyloseq packages in R (Love et al., 2014). The DESeq2 package calculated centered log-ratio values for each taxon prior to analysis. We restricted the analysis to fungal species that were detected in at least eight unique samples. We used three predictors in the GLM: longitude, AI and MAT and we extracted the model coefficients for the two climate variables on the log(2) scale. Again, we analysed AM-associated and EM-associated tree genera separately. We used the False Discovery Rate to adjust *p*-values for multiple comparisons (Benjamini & Hochberg, 1995) after excluding taxa who fell below a base abundance threshold (using the independent Filtering process in the DESeq2 package).

In addition to inferring individual fungal species associations, we also used the coefficients of the differential abundance models in a

meta-analysis. We refer to this as a meta-analysis since the outputs of one set of models (the differential abundance tests) were used as data for a second model. We used the log(2)-fold change coefficients as dependent variables in a linear model with fungal guild as the independent variable and the regression weighted by the inverse of the standard error of the coefficient estimate. This was performed for coefficients for the association with AI and MAT, separately for the set of AM and EM tree samples.

2.7 | Question 2: What aspects of fungal communities correlate with seedling performance under contrasting climate conditions?

We used the data on seedling performance in our field plantings and their root fungal communities to answer this question. We used stem diameter as our metric of performance as stem height was highly determined by deer browsing. As before, we analysed AM-associated and EM-associated seedlings separately. Within each mycorrhizal type, we first built one linear mixed model with all seedlings and field site (northern vs. southern), rainfall treatment (ambient vs. reduced) and their interactions with the relative abundance of three fungal guilds (NME, saprotrophs and either arbuscular or EM fungi as appropriate to the seedling group). We modelled fungal guild relative abundance with linear and quadratic terms to allow for non-linear relationships with seedling performance. Initial seedling size and year planted were included as fixed covariates and seedling species as a random effect. If statistically significant interactions were found between fungal guild abundance(s) and sites or treatments, sub-models were run within sites and treatments to investigate the nature of the interaction. A similar set of linear mixed models was fit using fungal guild diversities as predictor variables.

To explore the potential individual taxa impact on seedling performance, we used a combination of two high-dimensional regression approaches. We used LASSO (least absolute shrinkage and selection operator) to fit predictive models of seedling performance in each of the eight combinations of field site (northern vs. southern), rainfall treatment (ambient vs. reduced) and seedling mycorrhizal type (AM vs. EM) using the relative abundance of all fungal taxa present in at least eight (AM seedlings) or five (EM seedlings) samples. We used different frequency thresholds due to the different sample sizes of AM versus EM seedlings to retain a similar frequency (i.e. only fungal taxa detected in at least 10% of samples were used as predictors in the model). The LASSO regularization algorithm takes models with many predictors and shrinks coefficients to zero as necessary to achieve the set shrinkage threshold, λ (Tibshirani, 1996). We used the LOPR function in the HDCI package to provide less-biased estimates of the coefficients for LASSO retained predictors along with bootstrapped confidence intervals (Liu et al., 2017). Fungal taxa that were retained by LASSO and had bootstrapped confidence intervals not crossing zero were considered strong candidates for beneficial or detrimental interactors with tree seedlings in a specific climatic context.

Additionally, we used ridge regression, again implemented in the LOPR function, with bootstrapped confidence intervals to make a separate set of predictive models for the eight data subsets. Ridge regression differs from LASSO in that it shrinks regression coefficients toward but never goes to zero to meet the shrinkage threshold. Thus, Ridge regression does not perform model selection (removal of predictors) but does provide a relative (albeit biased) measure of association between all original predictors and the outcome variable that accounts for the intercorrelation among predictors (Hoerl & Kennard, 1970). We first determined the predictive ability of the best Ridge regression model in each of the eight 'mycorrhizal type × climate condition' combinations by performing five rounds of training on a random 80% of samples and testing on the remaining 20%. Models that can effectively predict seedling performance in the test dataset give confidence that fungal community composition contains information related to seedling performance. We then used the coefficients of Ridge regression models to provide a metric of impact for all fungal taxa on seedling performance in contrasting environments, with the coefficients shrunk toward zero based on their impact on model predictive ability (i.e. taxa whose relative abundance are most predictive of seedling growth retain coefficients of large magnitude, while those that contribute less to prediction have their coefficients shrunk toward zero while maintaining their sign). Rather than interpret these coefficients individually, as they may be highly biased and unstable, we instead used them in a meta-analytic approach to determine if taxa from different fungal guilds differed in their average impact on seedling performance. We used a sign-preserving log-transform (log-modulus transformation) of the ridge regression coefficients as a dependent variable in a linear model with fungal guild as the independent variable and the inverse of the bootstrapped confidence interval as weights (John & Draper, 1980).

We used both LASSO and Ridge regression models as each served distinct purposes in our analysis. The LASSO regression provides a better approach for identifying individual fungal taxa with consistent, strong effects on seedling growth as it performs both model selection and parameter estimation, removing predictor variables (fungal taxa) with weak effects. We used Ridge regression to determine whether groups of taxa (functional guilds) differed on average in their predicted effect on seedling growth. For this purpose, we did not want to eliminate predictors but rather estimate a regression coefficient (no matter how weak) for each taxa for use in the meta-analytic comparisons among functional guilds.

2.7.1 | Candidate fungal taxa for climate niche expansion of host trees

Finally, we combined the evidence gathered from all the above analyses to select candidate fungal taxa to highlight. To be considered a candidate taxon, we looked for a fungal taxon that was (1) significantly correlated with one or more climatic variables in our differential abundance analysis and (2) retained as a consistent predictor of seedling growth in the relevant climate

conditions in LASSO models, with climate represented via our two sites (southern vs. northern) and the rainfall reduction experiment in each site.

3 | RESULTS

3.1 | Question 1: How do root-associated fungal communities vary across climatic gradients?

In our geographic dataset, we were able to identify ~77% of sequence reads per sample on average to a guild. In our seedling growth dataset, we were able to assign 63% and 73% of sequences for AM and EM seedlings, respectively. Our empirical results are similar to most studies using general fungal ITS primers, in that we recovered a very low level of AM fungal sequence reads; thus, our data underestimate the relative abundance and diversity of Glomeromycotan fungi (Lekberg et al., 2018). In our dataset, NME were overwhelming Ascomycotan (98.3% of the sequences and 86% of the fungal taxa annotated to this phylum). Most of the taxa in this group come from genera in four dominant orders: Heliotales (31%), Hypocreales (16%), Capnodiales (16%) and Pleosporales (10%). The top families included Nectriaceae, Cladosporiaceae, Myxotrichaceae, Heliotales (incertae sedis) and Herpotrichiellaceae.

3.1.1 | Relative abundance and diversity of fungal guilds across climatic gradients

The relative abundance of fungal guilds varied across both aridity and temperature gradients for both AM- and EM-associated tree species. NME increased in relative abundance with drier (lower AI) and warmer (higher MAT) sites (Figure 2; Table S2). This occurred across both AM and EM tree species but was quantitatively stronger in AM compared to EM species. This pattern appeared very strongly in the genus *Acer* but was also evident in the other AM tree species analysed collectively (Table S3). It was not individually evident in the genera *Quercus* or *Carya* when analysed alone, but it was present in the mix of other EM trees (consisting of Betulaceae, *Populus*, *Tilia* and *Pinus* samples, Table S3). The relative abundance of AM fungi was unrelated to climate gradients. Note that our methods likely underestimate the abundance and diversity of AM fungi. However, EM fungal relative abundance increased in wetter sites (higher AI) for EM-associated trees (Figure 2; Table S2). This pattern was individually significant for the genus *Quercus* analysed alone, marginally so for the genus *Carya*, but not for the mix of 'other' EM tree species (Table S3).

Overall fungal diversity was positively associated with MAT and negatively associated with longitude (i.e. higher in western sites) but did not vary with aridity (Table S4). The diversity of NME fungi responded differently to MAT for AM versus EM tree species—increasing with MAT in AM trees but flat/decreasing in EM trees (Table S4). AM fungal diversity showed no relation to climate gradients. Finally, EM fungal diversity was higher in Midwestern compared to eastern locations, even after controlling climate variables (Table S4).

3.1.2 | Individual fungal taxa responses to climatic gradients in AM-associated tree species

In AM-associated plants, six fungal taxa had significant associations with drier sites, including two NME, three saprotrophs and one EM fungus (likely a superficial component of the root community). Seven taxa had significant associations with wetter sites, including soil saprotrophs, an EM fungus and a taxon from the 'other' grouping (*Papiliotrema flavescens*). Six taxa were associated with colder sites, and five with hotter sites. One taxon, *Cladosporium* sp., was associated with both hotter and drier locations, while one other (*Solicoccozyma terricola*) was associated with both colder and wetter locations (Figure 3; Table S5).

More broadly, fungal species from the roots of AM-associated trees showed substantial differences in their average responses to aridity between guilds (ANOVA, $p = 0.016$). NME, as a group, tend to be positively associated with aridity (mean = −0.341, 95% CI = −0.554 to −0.127; Figure 3; Table S6). Of the other guilds, only the 'other' group had an average response that differed significantly from zero (mean = 0.365, 95% CI = 0.005–0.725; Figure 3; Table S6).

With respect to MAT, guild was not a significant predictor of fungal taxa responses (ANOVA, $p = 0.497$). However, the non-mycorrhizal endophyte guild did show an average response significantly different from zero (mean = 0.339, 95% CI = 0.091–0.587). No other guild differed on average from zero (Figure 3; Table S6).

3.1.3 | Individual fungal taxa responses to climatic gradients in EM-associated tree species

In EM-associated roots, only two fungal taxa were associated with drier locations and two with wetter locations (Figure 4; Table S5). On the contrary, seven taxa were associated with colder locations, while only two were associated with hotter locations (Figure 4). Of the seven, four were known EM fungi, while a fifth (*Meliniomyces*) is known to associate with the roots of ericoid and EM plants and can sometimes, but not always, form EM structures (Agerer, 1987). The two associated with hotter locations were both soil saprotrophs (Figure 4; Table S5).

Fungal guild was a significant predictor of individual fungal taxa responses for AI and MAT for fungi on roots of EM associate trees (Figure 4; Table S6). For AI, soil saprotrophs on average displayed negative responses (associated with drier sites, mean = −0.2085, 95% CI = −0.371 to −0.039). The average EM fungal taxa response was marginally significantly greater than zero (associated with wetter sites, mean = 0.261, 95% CI = −0.010 to 0.532). For MAT, the average EM fungal response was significantly negative (associated with colder sites, mean = −0.823, 95% CI = −1.133 to −0.514).

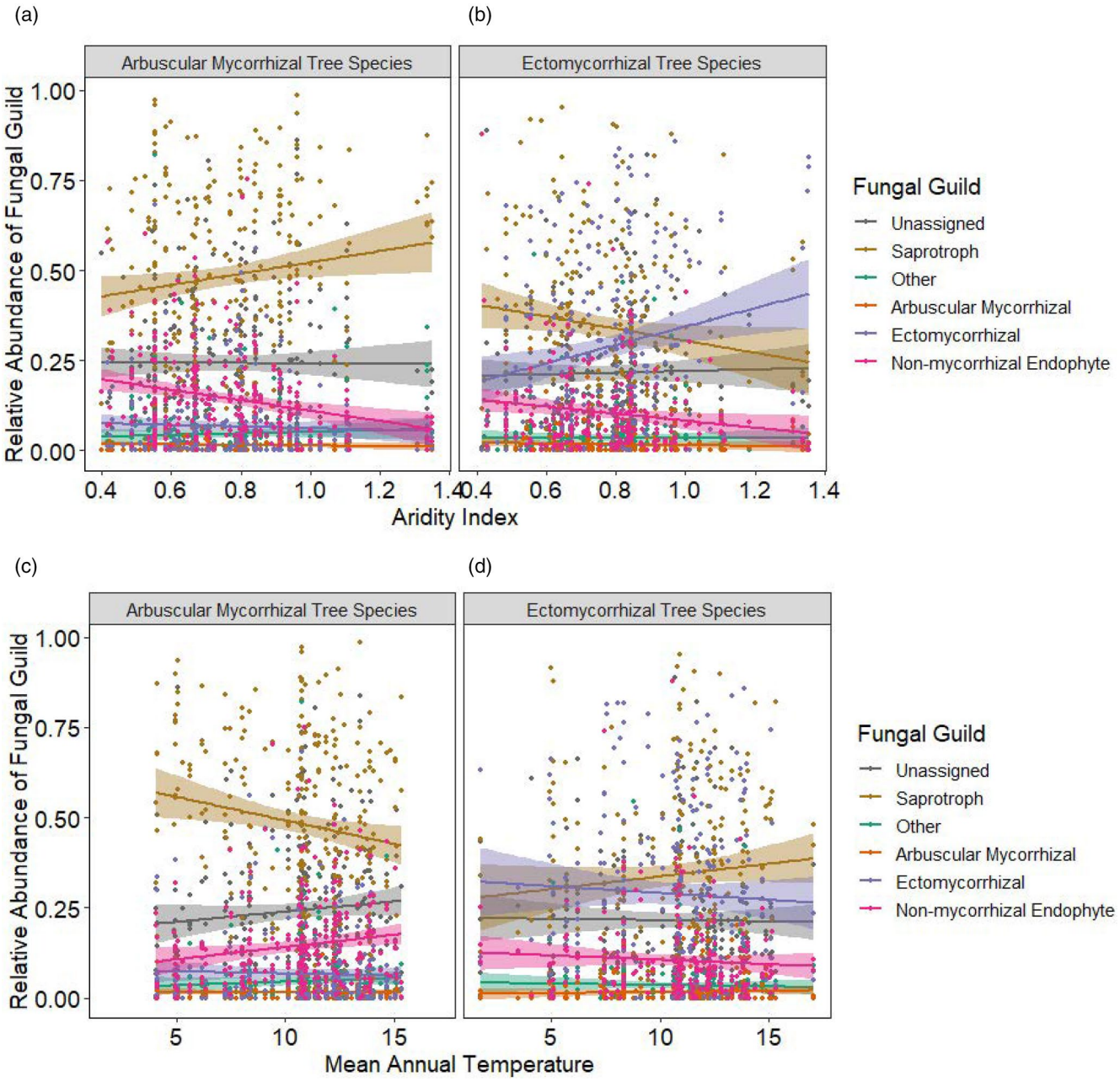

FIGURE 2 Relative abundance of six fungal guilds versus (a, b) Aridity Index (smaller numbers = drier sites) and (c, d) Mean annual temperature, separately for arbuscular mycorrhizal-associated tree species (a, c) and ectomycorrhizal-associated tree species (b, d). Note that proportional relative abundances are compositional (add to 1), so changes in one guild are not independent of changes in other guilds. See Section 2 for description of guild assignments.

3.2 | Question 2: What aspects of fungal communities correlate with seedling performance under contrasting climate conditions?

3.2.1 | Correlations of fungal guild relative abundance and diversity to seedling performance

Across both sites and rainfall treatments, the final stem diameter of AM-associated seedlings after 3 years of growth displayed a positive linear relationship with the relative abundance of AM fungi, and quadratic (hump-shaped) relationships with the relative abundance of soil saprotrophs and NME (Figure 5; Table S7). These patterns were all stronger at the central Illinois site compared to the northern Wisconsin site (Figure 5; Table S7). These relationships did not interact significantly with the rainfall reduction treatment (Table S7). For AM-associated seedlings, higher stem diameter was also associated with greater AM fungal diversity. This was marginally stronger at the southern versus northern site (Table S8).

For EM tree seedlings, there were no significant relationships with fungal guild relative abundance across both sites and

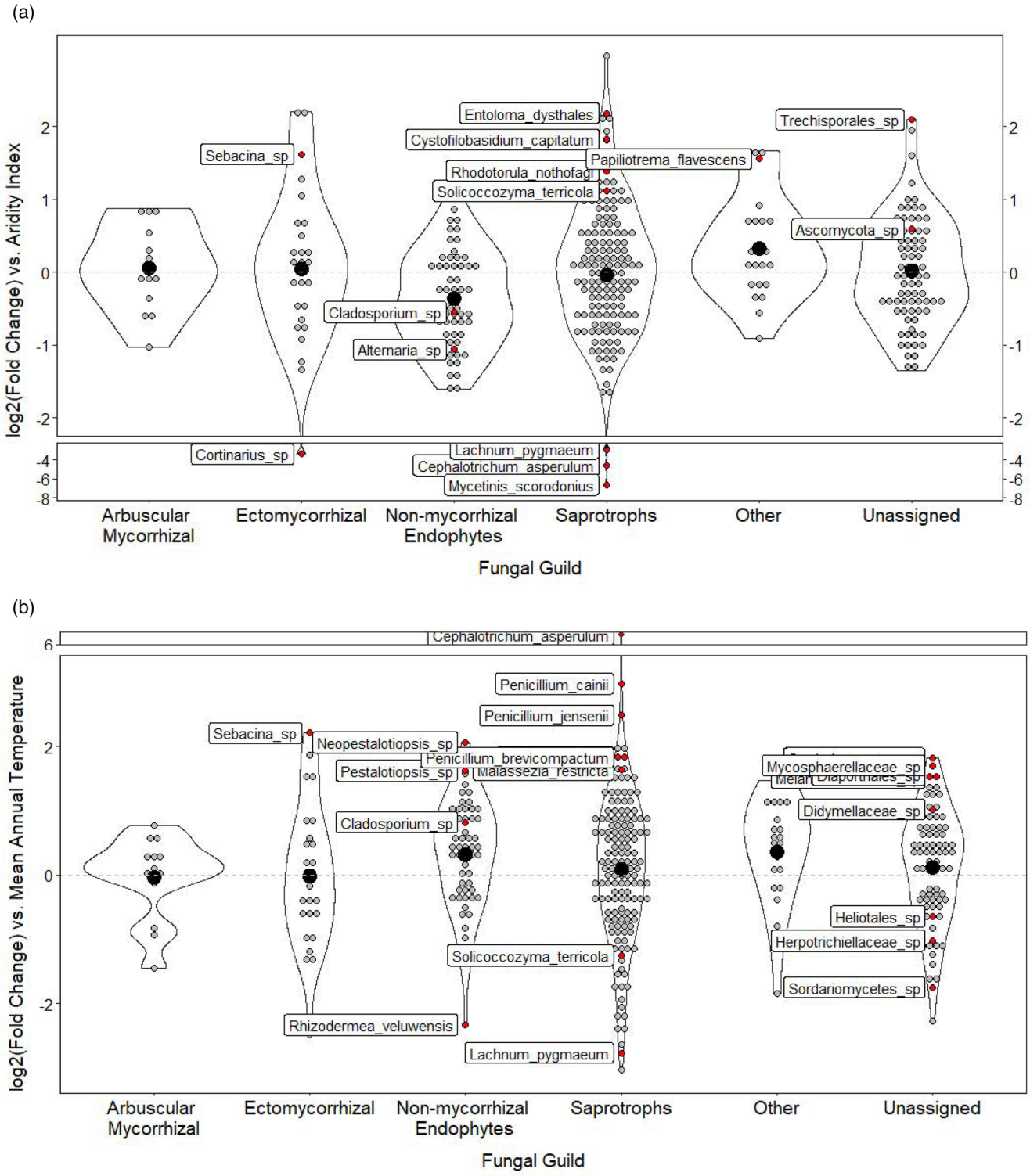

FIGURE 3 Violin plot of $\log_2$-fold changes (coefficients of a generalized linear model) for fungi on roots of arbuscular mycorrhizal-associated trees. Named taxa had significantly different coefficients from 0 after using a false discovery rate correction ($p < 0.05$). (a) Associations with Aridity Index (negative values = associated with dry climates, positive values = associated with wet climates). (b) Associations with mean annual temperature.

rainfall treatments (Table S7). However, stem diameter displayed contrasting relationships with EM fungal diversity: seedling stem diameter was positively correlated with EM fungal diversity in the ambient rainfall conditions but not in the reduced rainfall conditions. This was primarily evident in the southern site (Table S7).

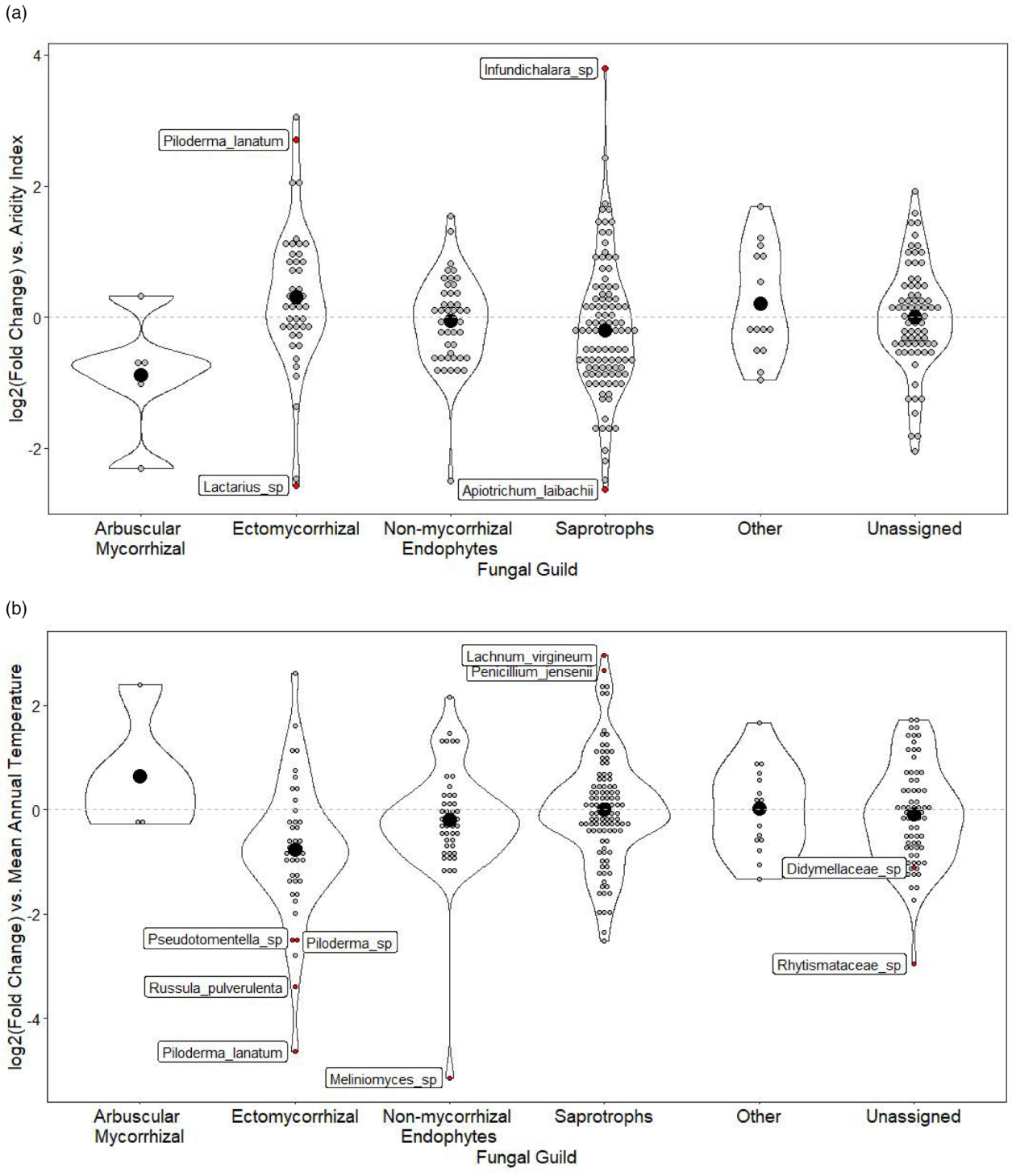

FIGURE 4 Violin plot of $\log_2$-fold changes (coefficients of a generalized linear model) for fungi on roots of ectomycorrhizal-associated trees. Named taxa had significantly different coefficients from 0 after using a false discovery rate correction ($p < 0.05$). (a) Associations with Aridity Index (negative values = associated with dry climates, positive values = associated with wet climates). (b) Associations with mean annual temperature.

3.2.2 | Associations of individual fungal taxa to seedling performance for AM-associated species

For AM-associated seedling species at the southern site, LASSO models in the rainfall reduced treatment retained three fungal taxa as consistent predictors of greater stem diameter: two NME (*Cladosporium* sp. and *Mycosphaerella latebrosa*) and an unidentified AM fungal taxon in the order Paraglomerales. All three had positive associations. In the ambient rainfall conditions, LASSO models retained four taxa, again all positively associated with seedling

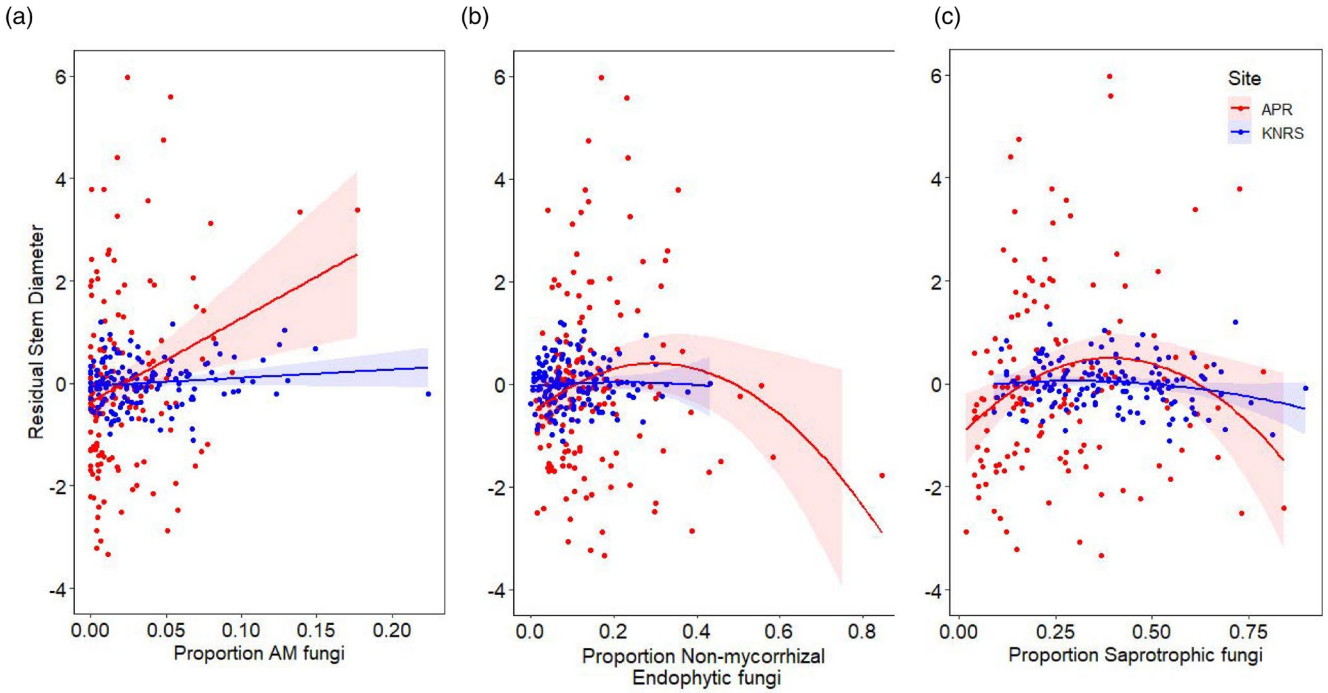

FIGURE 5 Residual stem diameter (stem diameter residuals from model adjusting for seedling species, planting year and initial height) for arbuscular mycorrhizal (AM)-associated seedling species versus the relative abundance of (a) AM fungi, (b) non-mycorrhizal endophytic fungi and (c) saprotrophic fungi in our southern field site (red, central Illinois) and our northern field site (blue, northern Wisconsin). Trend lines represent (a) linear regressions ($R^2 = 0.063$, 0.015 for APR and KNRS sites, respectively), (b) quadratic regressions ($R^2 = 0.040$, 0.002 for APR ad KNRS sites, respectively) and (c) quadratic regressions ($R^2 = 0.053$, 0.028 for APR and KNRS sites, respectively. For statistical inference, see Table S7. Red text = southern field site, blue text = northern field site.

diameter. These included 1 AM fungus (*Rhizophagus* sp.), one soil saprotroph (*Paramyrothecium* sp.) and two fungi in the 'other' category (*Nectriopsis exigua*, a mycoparasite and *Metarhizium marquandii*, an animal parasite). No individual fungal taxa were retained in LASSO models attempting to predict seedling stem diameter at the northern site.

Ridge models for AM-associated seedlings in the four climate conditions had varying predictive power. Ridge models had some predictive power in both rainfall treatments in the southern site, but little predictive power at the northern site (see Table S9). Ridge regression coefficients varied strongly by lifestyle guild in three of the four climate conditions (ANOVA, $p < 0.0001$ for both reduced and ambient rainfall treatments at the southern site, and for rainfall reduced conditions at the northern site; $p = 0.12$ for ambient conditions at the northern site, Table S10). In the rainfall reduced conditions at the southern site, NME on average had positive associations with seedling stem diameter (mean = 0.800, 95% CI = 0.336–1.263; Figure 6). EM fungal taxa had on average negative associations (mean = −0.803, 95% CI = −1.364 to −0.243; these fungi were likely present superficially on the root surface and not symbiotic with these seedlings). However, in the ambient rainfall conditions, soil saprotrophs on average had positive associations (mean = 2.00, 95% CI = 1.404–2.605; Figure 6). Soil saprotrophs also had on average positive associations at the northern site in rainfall reduced conditions (mean = 1.086, 95% CI = 0.616–1.555; Figure 6).

3.2.3 | Associations of individual fungal taxa to seedling performance for EM-associated species

For EM-associated seedlings, LASSO models only retained individual predictors for rainfall reduced treatments at the northern site. Retained predictors included the total relative abundance of NME, one soil saprotroph (*Geoglossum* sp.) and an unidentified member of the Herpotrichiellaceae. Ridge models were highly variable in their predictive power due to low sample sizes in each climate combination but had the highest average predictive power in the rainfall reduced treatment at the northern site (Table S9). Ridge regression coefficients differed among lifestyle guilds for three of the four climatic conditions (southern site, rainfall reduced: $p < 0.0001$; southern site, ambient: $p = 0.043$; northern site, rainfall reduced: $p = 0.003$; northern site, ambient: $p = 0.225$; Table S10). EM fungal taxa, on average, had positive associations with seedling stem diameter at the southern site in both rainfall conditions and in the ambient rainfall conditions at the northern site, but on average negative associations in the rainfall reduced conditions at the northern site. Soil saprotrophs had on average positive associations with seedling stem diameter in the southern site in rainfall reduced conditions, while NME had, on average, negative associations with seedling stem diameter in ambient conditions at the southern site (Figure 6).

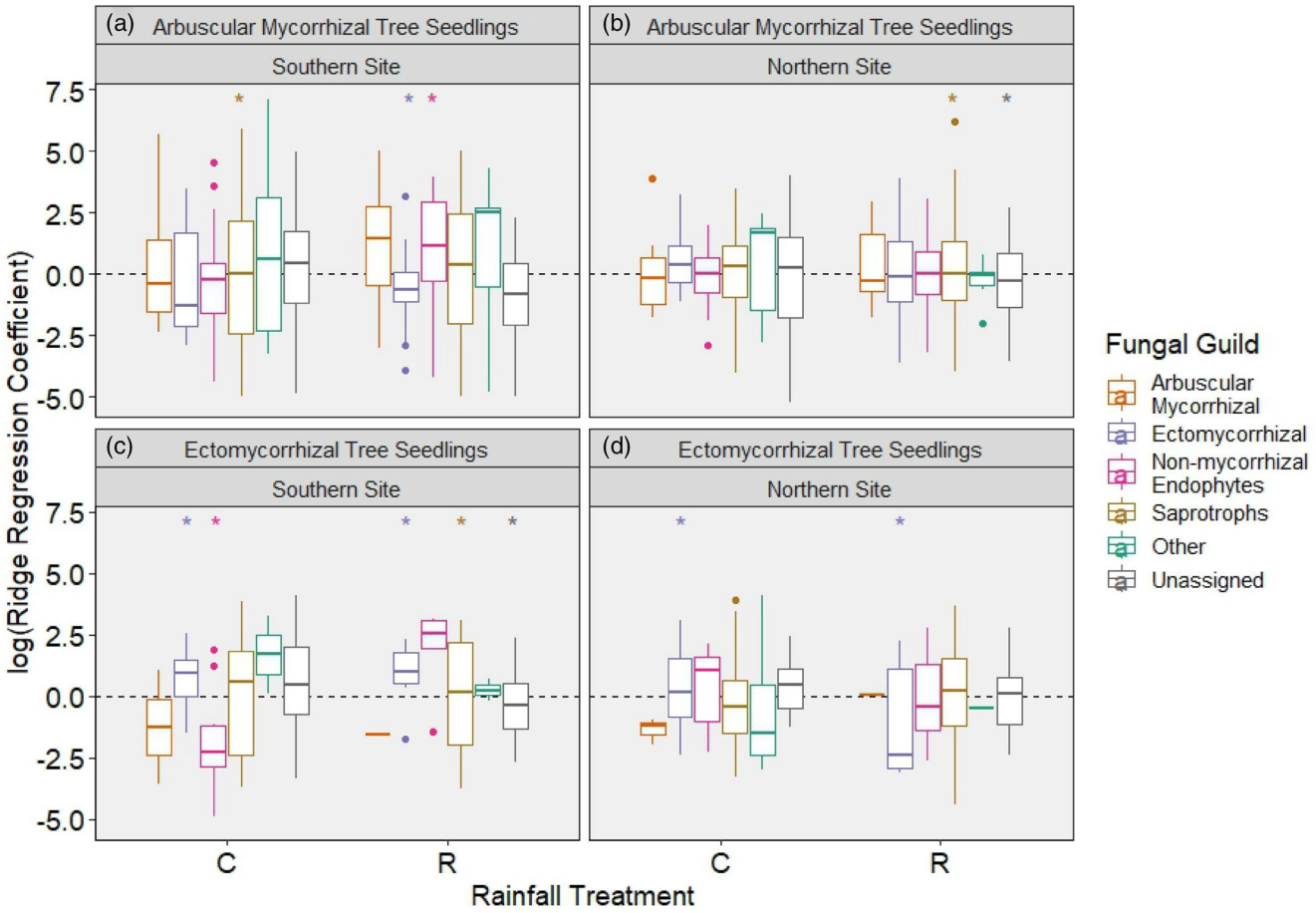

FIGURE 6 Estimates (log-modulus transformed) of the effects of individual fungal taxa relative abundance on seedling stem diameter from Ridge regression, averaged by fungal guild for southern (a, c) and northern (b, d) sites, by mycorrhizal type (a, b = arbuscular mycorrhizal, c, d = ectomycorrhizal) and rainfall treatment (C = ambient, R = reduced). *Fungal guilds with means significantly different from zero.

3.2.4 | Candidate fungal taxa for climate niche expansion of host trees

Of the 2302 unique fungal taxa (identified to named species or higher-level taxonomic ranks) in the geographic dataset, 1348 of those taxa (59%) were also present in the field experiment dataset. This subset of overlapping fungal taxa covered 91% of the total sequence reads in the geographic dataset and 98% of the total sequence reads in the field experiment dataset.

The genus *Cladosporium* had the strongest evidence base to support a role as a niche broadening agent. *Cladosporium* was one of only two taxa significantly associated with both drier and hotter locations (Figure 3). It was also retained as a consistent and reliable predictor of seedling growth in the hottest, driest conditions using LASSO regression. *Cladosporium* relative abundance was significantly correlated with both climatic gradients (AI and MAT) using only samples from a single tree genus (*Acer*, the most abundantly sampled genus—$p = 0.03$ and <0.0001, respectively—Figure 7). Furthermore, its relative abundance was strongly positively correlated with *Acer* seedling stem diameter in our hot, dry southern site ($R^2 = 0.38$, $p < 0.0001$; Figure 7) but not in our cooler, wetter site ($p = 0.58$; Figure 7). Note that, consistent with our geographic analysis of mature trees, the abundance of *Cladosporium* was substantially lower on seedlings at our northern site compared to our southern site. This weakened our ability to detect significant relationships between *Cladosporium* relative abundance and seedling performance at the northern site.

In our dataset, we had 40 non-singleton ASVs assigned to the *Cladosporium* genus, with the classifier unable to make any finer identifications. However, of the 64,243 sequence reads assigned to *Cladosporium*, >80% came from a single ASV and >97% came from the top five ASVs. When compared to the UNITE fungal database via BLAST, the five top ASVs all had best hits to the same three species: *C. cladosporioides*, *C. herbarum* and *C. delicatulum* (all 100% identity to the top ASV).

An additional limitation in our approach is that for a fungal specific taxon to meet all our criteria for being considered a candidate 'range expander', it would need to present (and be reasonably common) in both our geographic dataset and in our field experiment, which only occurred at two sites. While there were surely many fungal taxa important to the Eastern Temperate Forest biome that were not present in our two field sites, we did have substantial overlap between the data sets, especially for common and frequent taxa.

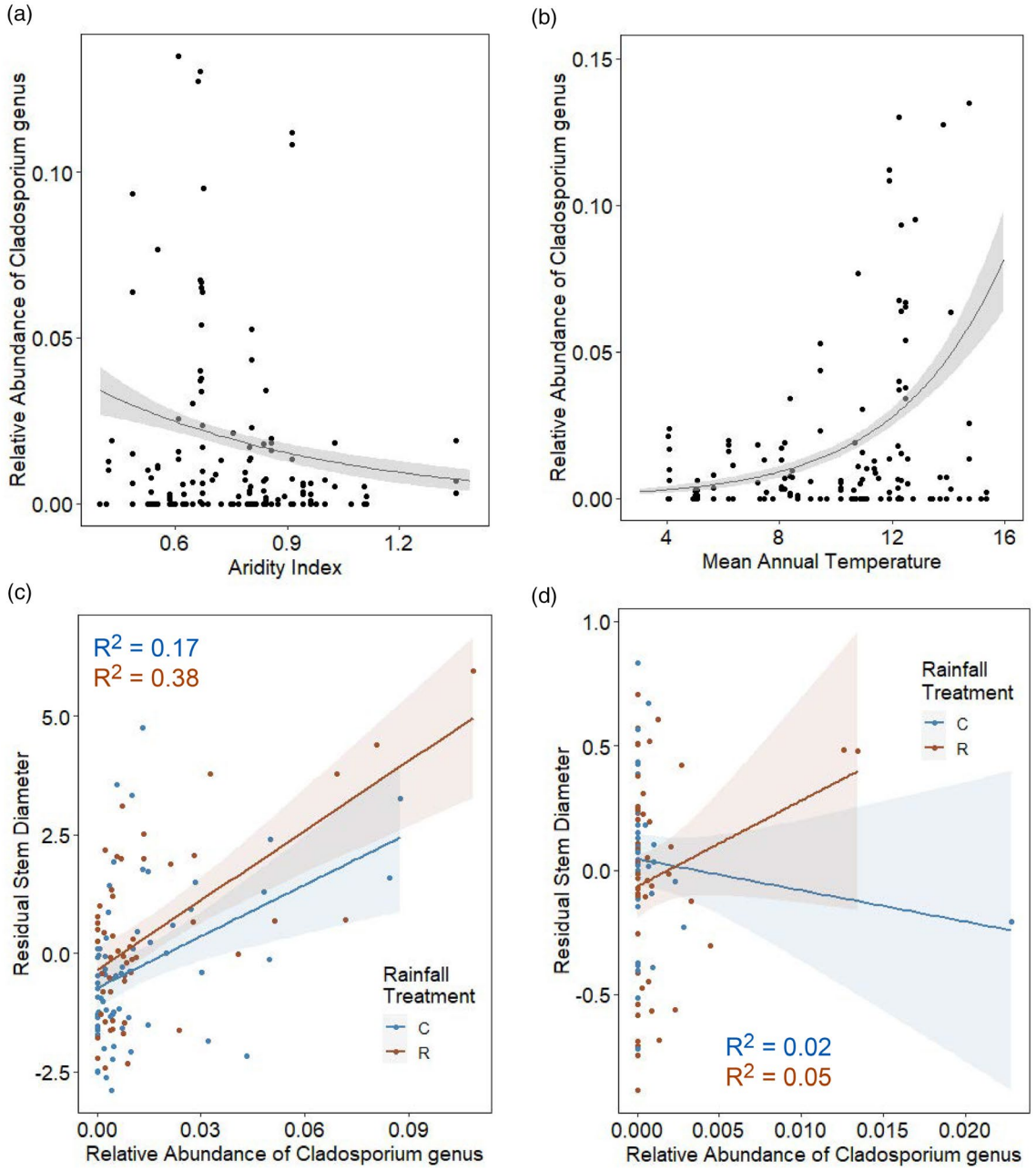

FIGURE 7 The *Cladosporium* genus is a candidate for a potential agent expanding the niche of *Acer* trees in hot, dry conditions. The relative abundance of *Cladosporium* sp. on *Acer* roots increases with (a) greater aridity (lower Aridity Index) and (b) higher temperatures. Seedling performance increased with increasing *Cladosporium* relative abundance at the southern field site (c) but not the northern site (d). R^2 values refer to simple linear regressions—for statistical inference, see Table S7. Maroon text = reduced rainfall treatment, blue text = ambient rainfall treatment.

4 | DISCUSSION

If tree populations rely on associating with varying fungal taxa to broaden their environmental niche breadth, then we predicted that variation in root-associated fungal community structure would correlate with specific climatic gradients both between and within tree species. We found that the relative abundance of fungal guilds varied strongly across climatic gradients. Specifically, we found that non-mycorrhizal endophytic fungi (NME) were increasingly dominant on roots from hotter and drier locations, especially for AM trees, both for the guild as a whole and within individual fungal endophyte taxa. Additionally, we predicted that at least some of the fungal guilds and taxa that vary across climate gradients would also positively associate with seedling growth in the corresponding climate condition. Here, we found that seedling growth in hot, dry conditions was greatest with higher relative abundance of AM fungi but intermediate relative abundance of NME. Several individual endophytic taxa were strong predictors of seedling growth in hot, dry conditions.

Most notably, the genus *Cladosporium* emerged as a strong candidate taxon to be contributing to the tolerance of *Acer* trees to hot, dry conditions.

4.1 | How does the composition and diversity of root-associated fungal communities vary across climatic gradients?

Our results are consistent with other large-scale surveys of fungal communities across continental and global scales, although other surveys have measured fungal communities in bulk soils rather than in plant roots directly. A meta-analysis of large-scale soil fungal surveys similarly found that climate variables (temperature and precipitation) were the most important determinants of individual fungal taxa distributions, followed by soil and vegetation metrics. EM fungal richness (and individual EMF taxa) tended to increase with colder temperatures while plant 'pathogens' had wider climatic tolerances (Tedersoo et al., 2014; Větrovský et al., 2019). Other surveys of soil have indicated stronger roles of soil properties, especially pH and dispersal in shaping beta-diversity (Talbot et al., 2014). A continental survey of specifically rhizosphere fungi of trees of the Populus genus similarly found climate variables as the strongest determinants of fungal community structure, with EM fungal abundance and diversity increasing in cooler climates (Van Nuland et al., 2023).

4.2 | What aspects of fungal communities correlate with seedling performance under contrasting climate conditions?

We found no evidence that general fungal or NME diversity was associated with greater host growth in stressful environments; instead, we found associations between host growth and either AM or EM fungal diversity. We found more consistent evidence that the turnover of fungal taxa (or functional guilds) across space could be contributing to host range expansion, since we found some examples of individual taxa (e.g. *Cladosporium*) and functional guilds (e.g. NME) that both increased in abundance along climate gradients and were positively associated with tree seedling growth under contrasting climates.

Despite caveats, broad patterns in our two datasets suggest that an increased abundance of NME in or on roots may be adaptive for trees in hot, dry conditions. In our southern field site, which was both hotter and more arid in summer months compared to the northern site, seedling growth displayed a hump-shaped relationship with the non-mycorrhizal endophyte guild. Seedling growth increased up to around ~25% relative abundance of the guild, after which growth plateaued or decreased. This may represent multiple processes; for instance, these endophytic fungi exert a cost as well as any benefit, and there may be a certain fungal load which costs overwhelm benefits even in stressful conditions. Additionally, the change in non-mycorrhizal endophytic guild relative abundance may also reflect a change in the composition of the root community. On average, most fungal species assigned to the NME group were more relatively abundant on tree roots in hot and dry locations. Thus, the overall increase in this NME group stemmed from correlated responses of many individual species, not just a few highly dominant ones. Similarly, when considering the coefficients for individual fungal taxa from Ridge regression, the NME group showed on average positive correlations with seedling performance (stem diameter) in our hottest, driest condition.

Although most NME in our dataset would be considered pathogens, we rarely found evidence that increased relative abundance of fungi in this group correlated with reduced plant growth—in fact, on average, they showed positive correlations, especially in drought-stressed seedlings. However, the assignment of fungi to functional guilds may be error-prone (Marc-André et al., 2018). Many fungi can vary along a parasitism-beneficial endophytic spectrum within genera, within species and even within genetically distinct strains based on environmental conditions or plant hosts (Geisen et al., 2021; Porras-Alfaro & Bayman, 2011; Redkar et al., 2022).

The *Cladosporium* genus stood out as a promising candidate group to explore as potential mediators of tree drought/heat tolerance and niche expansion. The *Cladosporium* genus was the only taxon that met the criteria for a candidate taxon. First, it was both abundant and frequent for AM tree species in our geographic survey. Second, its relative abundance on AM tree roots was positively associated with both hotter and drier conditions. Finally, it was a strong and consistent predictor of seedling stem diameter in the hottest and driest conditions of our field study. These patterns were present within the AM trees as a group, but also within the genus *Acer* specifically.

The genus *Cladosporium* is highly diverse, including >700 described names, among several species' complexes and distributed worldwide in many environments (Bensch et al., 2015; Dugan et al., 2004). As a plant endophyte, it includes known pathogens (e.g. *C. fulvum*, causal agent of tomato leaf mould disease) but is also often isolated from healthy plants (Bensch et al., 2015), including temperate tree species (Marčiulynas et al., 2022; Paul & Yu, 2008). Isolates of *C. cladosporioides* in particular have been found to increase total plant biomass (Andreo-Jimenez et al., 2023), root biomass under drought (Qin et al., 2016) and seed germination (Ndinga-Muniania et al., 2021). The mechanisms of plant growth promotion and enhanced drought response are unclear but could involve increased osmolyte production (Dastogeer et al., 2018).

Mycorrhizal fungi also benefited plant growth, but in less clearly climate-specific ways. This is consistent with the understanding of mycorrhizal fungi as mediating nutrient acquisition more than climate tolerance (Smith & Read, 2008). Although AM fungal relative abundances and diversity lacked correlation with any climatic variables, it is likely that we missed true relationships between specific AM fungal taxa and climate gradients since our

data likely underestimated the relative abundance and diversity of Glomeromycotan fungi (Lekberg et al., 2018). Thus, our results should not be interpreted to indicate that AM fungal taxa do not respond to climatic variation. However, our methods were sufficient to detect a positive association between the relative abundance of AM fungi and seedling growth in our southern field site as well as specific positive impacts of individual AM fungal taxa on seedling growth.

The overall relative abundance of EM fungi showed the opposite pattern to NME: increased abundance in wetter sites, although this was not evident in many individual taxa. And while the overall abundance of EM fungi was not related to temperature, several individual taxa were more abundant in colder locations, while no individual EM fungal taxa were more abundant in warmer locations. Increased association with EM fungi in wetter sites may reflect changing needs of host plants—in areas less limited by water, trees may become more limited by nutrients and thus benefit from additional investment in mycorrhizal relationships. Similarly, in colder locations, specific EM fungi with strong decomposer abilities may be beneficial when decomposition rates are slowed by low temperatures (Steidinger et al., 2019). Two of the four EM fungi significantly associated with colder climates were from the genus *Piloderma*, which can access organic nitrogen for their host plants (Heinonsalo et al., 2015). While the overall relative abundance of EM fungi was not correlated with seedling growth at either of our field sites, we did find that on average the individual effect of EM fungi tended to be positive for seedling performance, especially in ambient rainfall conditions, at both of our field sites. This suggests that EM fungal taxa may benefit host trees most when climate conditions are less stressful, making plant productivity more likely to be limited by nutrient acquisition. Unfortunately, we have little direct data on soil nutrient conditions at our sampled sites. Future analyses will be necessary to determine how mycorrhizal abundance, diversity and composition relate to non-climatic gradients across tree species ranges (such as soil orders, nitrogen deposition and fire frequency).

Climatic niche breadth may reflect not only intrinsic tree traits but also their varying relationships with rhizosphere microbes. NME present an intriguing and understudied group of root fungi whose impact on tree health and ecology is still poorly understood. While the correlative approaches employed in this study cannot conclusively determine whether these fungi are indeed allowing for an expanded climate niche for their host trees, combining data on geographic distributions and seedling growth in contrasting climates identified potential candidates for future experimental investigations. While our study focused on tree-fungal interactions, it is important to note that tree interactions with other microbial groups (bacteria, archaea, nematodes, etc.) might also contribute to tree range sizes or responses to climate change. Understanding if, and how, tree climate niches are affected by geographically varying microbial associations may prove vital to accurately predicting the vulnerability of tree species to changing climates. Additionally, identifying specific tree-fungal interactions that can promote tree tolerance to stressful climates may offer new avenues for the management and restoration of forest ecosystems in the future.

AUTHOR CONTRIBUTIONS

Cassandra M. Allsup and Richard A. Lankau conceived of and designed the study, with contributions from Kathleen Thompson and Isabelle George. Cassandra M. Allsup recruited and organized volunteer participants and led information sessions with volunteer groups. Kathleen Thompson organized sample transport, data management and communication with volunteer collectors. Isabelle George, Kara Fontana, Shayden Fisher and Chloe Hansen performed laboratory research. Cassandra M. Allsup, Isabelle George and Kathleen Thompson performed field experiments. Isabelle George, Kathleen Thompson and Cassandra M. Allsup designed and maintained the project website. Ronald Li contributed to data analysis. Richard A. Lankau supervised undergraduate students in sample preparation and analysed the data. Richard A. Lankau wrote the manuscript with revision by all co-authors.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/1365-2745.70109.

DATA AVAILABILITY STATEMENT

Sequencing data for the volunteer collected samples is available in the NCBI Short-Read Archive under accession PRJNA1252726. Sequencing data for the experimental seedlings is available under accession BioProjects PRJNA944197, PRJNA944945, PRJNA944949 and PRJNA944953. Environmental and geographic metadata, data on field seedling performance and sequence counts for each fungal species are available at the Dryad depository at https://doi.org/10.5061/dryad.280gb5n2d (Lankau & Allsup, 2025).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Non-metric dimensional scaling ordination of fungal communities in the sterilized versus unsterilized comparison set, split out by tree mycorrhizal type.

Figure S2. Stacked bar chart of proportion of fungal sequences assigned to each fungal guild for arbuscular mycorrhizal (AM, left) and ectomycorrhizal (EM, right) tree species roots, that were either surface sterilized or not sterilized prior to DNA extraction.

Figure S3. Rarefaction curves of fungal species detection versus resampled sequence depth for the original set of 900 samples.

Table S1. List of woody genera indentified in our set of root samples.

Table S2. Linear mixed model results of fungal guild relative abundance (proportion of sequence reads) for arbuscular mycorrhizal (AM) and ectomycorrhizal (EM) tree species.

Table S3. Linear mixed model results of fungal guild relative abundance (proportion of sequence reads) separetly by tree genera.

Table S4. Linear mixed model results of fungal guild Shannon Weaver diversity index for arbuscular mycorrhizal (AM) and ectomycorrhizal (EM) tree species.

Table S5. List of fungal species (including unidentified species within genera) with significant associations with climate variables with adjusted $p < 0.05$ for differnetial abundance analysis.

Table S6. ANOVA of fungal taxa coefficients from differential abundance analyses by fungal guild, along with estimated marginal means for each guild.

Table S7. Linear mixed models of seedling diameter in the field experiment versus fungal guild relative abundance.

Table S8. Linear mixed models of seedling diameter in the field experiment versus fungal guild diversity.

Table S9. Summary of predictive power of ridge regression models for each combination of seedling mycorrhizal type, site, and rainfall treatment.

Table S10. ANOVA of ridge regression model coefficients for fungal taxa by fungal guild.

Appendix S1. Supplemental methods.

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