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RESEARCH ARTICLE

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Plant geographic origin and phylogeny as potential drivers of community structure in root-inhabiting fungi

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Abstract

1. Root-inhabiting fungal communities, including mutualists and antagonists, influence host plant performance, and can potentially shape plant community composition. However, there is uncertainty about how root-inhabiting fungal communities are structured, and if fungal community characteristics are significant predictors of host plant abundance.
2. In this study, we first assessed how root-inhabiting fungal communities were structured in relation to the phylogeny and geographic origins (native vs. exotic) of their host plants in an old-field community. In addition, we took into consideration the spatial arrangements (i.e. physical locations) of the individual host plants. We then tested if the relative abundances of pathogenic and beneficial arbuscular mycorrhizal (AM) fungi could predict host plant abundances.
3. We found that host plant phylogeny was an important factor in structuring the whole fungal community, irrespective of host plant origin. Furthermore, the spatial arrangements of individual host plants were a strong predictor of AM fungal community structure. Host plant phylogeny and spatial arrangements appeared to similarly affect the structure of pathogenic fungal communities. No distinct differences were observed between native and exotic plant species in fungal community characteristics. The relative abundances of AM and pathogenic fungi were not significant predictors for observed abundances of their host plants.
4. *Synthesis.* Host plant phylogeny and spatial arrangements can structure naturally occurring root-inhabiting fungal communities. The absence of distinct differences in fungal community composition, including pathogens, in exotic and native plants suggests long residence times and the consequent naturalization of exotic species in the region, allowing for the establishment of similar plant–microbial interactions between native and exotic species.

KEYWORDS
arbuscular mycorrhizal fungi, host plant abundance, host plant phylogeny, Illumina sequencing, pathogenic fungi, plant geographical origins, root-inhabiting fungi

1 | INTRODUCTION

Plant-inhabiting microbes are recognized as an integral component of their host plant. These microbes' effects on host plants can range from mutualistic to antagonistic, affecting nutrient acquisition (Jacoby, Peukert, Succurro, Koprivova, & Kopriva, 2017), growth (Compant, Clément, & Sessitsch, 2010; Hayat, Ali, Amara, Khalid, & Ahmed, 2010), and tolerance against stresses such as drought (Rodriguez et al., 2008; Ruiz-Lozano, Porcel, Bárzana, Azcón, & Aroca, 2012), salinity (Ruiz-Lozano, Porcel, Azcón, & Aroca, 2012), disease (Berendsen, Pieterse, & Bakker, 2012; Pozo, Jung, López-Ráez, & Azcón-Aguilar, 2010) and herbivory (Clay, Hardy, & Hammond, 1985; Koricheva, Gange, & Jones, 2009). Consequently, plant-inhabiting microbes can influence host plants' fitness and competitive ability (Aschehoug, Metlen, Callaway, & Newcombe, 2012; Clay, Marks, & Cheplick, 1993; Tkacz & Poole, 2015), which in turn can shape plant community composition (Afkhami & Strauss, 2016).

Fungi form a major group of plant-associated microbes in the root endosphere (i.e. fungi within roots, hereafter root-inhabiting fungi), including arbuscular mycorrhizal (AM) and pathogenic fungi. As a result, the ecological effects of plant–fungal interactions represent the net effect of positive interactions with AM fungi and the negative effects of pathogens. Although there has been much effort placed in trying to understand the physiological mechanisms involved in plant–root fungal associations, we have a limited understanding of how root-inhabiting fungal communities are assembled (Dickie et al., 2015). Contributing factors may include plant characteristics (Chagnon, Bradley, & Klironomos, 2015), host plant phylogeny (Tedersoo, Mett, Ishida, & Bahram, 2013; Wehner et al., 2014), soil characteristics (Dumbrell, Nelson, Helgason, Dytham, & Fitter, 2010; Wehner et al., 2014), spatial arrangement (i.e. physical locations) of host plants (Horn, Hempel, Verbruggen, Rillig, & Caruso, 2017), disturbance history (Vályi, Rillig, & Hempel, 2015) and stochastic processes (Dumbrell et al., 2010). Some of the factors are not mutually exclusive. For example, closely related plant species can share similar plant characteristics, such as morphology (Kembel & Cahill, 2005, 2011), production of secondary metabolites (Wink, 2003) and phenology (Davies et al., 2013), which can regulate colonization of root-inhabiting fungi (Wehner et al., 2014). Spatial arrangements of host plants can be autocorrelated with soil characteristics, such as pH and nutrient availability (Ritz et al., 2004). This complexity around the ecological factors involved in structuring root-inhabiting fungi makes it especially challenging to estimate their relative importance for any given plant community.

In addition, the relative contributions of these potential factors in shaping fungal communities in roots may depend on the types of fungi present. For instance, the degree of host specificity varies among fungal types; AM fungi can colonize a wide range of host plant species (Hart, Reader, & Klironomos, 2003), whereas specialist pathogenic fungi can only infect a group of plant species which are closely related (Gilbert & Webb, 2007; Parker et al., 2015). As such, host plant phylogeny can be more important in shaping specialist pathogenic than AM fungi in communities. Variability in dispersal ability of fungal taxa can affect spatial arrangements. For instance, AM fungi disperse via local hyphal growth and propagules, including spores, possibly limiting their dispersal to relatively short distances (Vályi, Mardhiah, Rillig, & Hempel, 2016). On the other hand, some fugal taxa can produce airborne spores, resulting in longer distance dispersal (Burge, 2002; Fierer et al., 2008). Thus, community structure of root-inhabiting fungi can depend on scales of host plant phylogeny and space in a given plant community.

Furthermore, the community structure of root-inhabiting fungi can be different between native and exotic plant species. This is because exotic plant species tend to be less responsive to soil antagonists and mutualists compared to native plant species, and may therefore exercise less control over the fungi colonizing their roots (Anacker, Klironomos, Maherali, Reinhart, & Strauss, 2014; Bennett & Strauss, 2013; Seifert, Bever, & Maron, 2009; Van Grunsven et al., 2007). This potential distinction in fungal communities and the level of responsiveness to fungi can be an important mechanism of invasion for exotic plants (Engelkes et al., 2008; Klironomos, 2002; Reinhart & Callaway, 2006). One hypothesis behind these observations is that invasive plant species have been released from natural enemies, including soil-borne pathogens, which control their populations in the native ranges (Inderjit & van der Putten, 2010; Mitchell & Power, 2003). Over time, however, exotic plant species, including invasive ones, may become more responsive to antagonistic microbes as pathogens can accumulate on exotic plants (Diez et al., 2010; Hawkes, 2007), leading to their decline (Flory & Clay, 2013). Recent studies showed the phenomenon of pathogen accumulation on invasive plant species over time (Mitchell, Blumenthal, Jarošík, Puckett, & Pyšek, 2010) and their decline (Flory, Bauer, Phillips, & Clay, 2017; Stricker, Harmon, Goss, Clay, & Luke Flory, 2016), including a case associated with soil-borne pathogens (Dostál, Müllerová, Pyšek, Pergl, & Klinerová, 2013, but see McGinn et al., 2017). Such dynamic temporal relationship between exotic plants and pathogens can contribute to maintain plant species diversity in communities (Bever, Mangan, & Alexander, 2015).

In this study, we investigated how communities of root-inhabiting fungi, including AM fungi and putative plant pathogens, were structured in relation to host plant phylogeny as well as the plant's geographic origins (native vs. exotic). Because root-inhabiting fungal community composition could be influenced by spatial autocorrelation, we took into consideration the spatial arrangements of individual host plants in the old field in order to isolate the effects of phylogeny and invasion status. We examined whole communities of fungi, as well as those of AM and pathogenic fungi, in roots of 142 individual plants across 10 native and 17 exotic plant species diverse in phylogeny via Illumina sequencing. We asked whether fungal community structure would be shaped by host plant phylogeny, or, alternatively, by the spatial arrangements of individual host plants. We also asked whether fungal communities would differ between native and exotic plants occupying the same plant community,

especially for pathogenic fungal communities in the context of the enemy release hypothesis. Because plant–microbe interactions are expected to have consequences for plant communities, we also explored whether AM and pathogenic fungi were significant predictors of plant species' abundances. We predicted that among the 27 co-occurring plant species, the relative abundances of pathogenic fungi in the roots would be negatively correlated with their host plant abundances, and that the opposite trend would be observed for AM fungi. Furthermore, we specifically identified root-inhabiting fungal families whose relative abundances were significant predictors of their host plant abundances via correlation analyses.

2 | MATERIALS AND METHODS

2.1 | Study site

The study site was located at the Long-Term Mycorrhiza Research Site (LTMRS, Klironomos, 2000) in the University of Guelph Nature Reserve, Guelph, Canada (43°32′30″N, 80°13′00″W). The site was established in 1996 in an old field which had not been disturbed since 1967. The soils were characterized as poor in nutrients, especially in phosphorus (Sherrard & Maherali, 2012). Total 40 permanent plots (1 m × 1 m) were established at regular intervals (5 vertical transects, 8 horizontal transects) within a 45 m × 50 m grid at the southern end of the site. Vertical transects were 10 m apart and horizontal transects were 5 m apart. The 40 permanent plots were used to census the plant community, and the immediate area surrounding the permanent plots within and in the vicinity of the 45 m × 50 m grid was used to collect plant specimens.

2.2 | Plant community survey

Prior to surveying vegetation, plots were hand raked to remove dead vegetation for better visibility. We used a point-intercept sampling method to survey vegetation in each 1 m^2 permanent plot (Yurkonis, Maherali, Bolton, Klironomos, & Newman, 2012). We sampled 81 points at 10 cm intervals in a 9 × 9 grid pattern, leaving a 10 cm buffer between the plot edge and the sampling grid. At each point, we suspended a weighted string from an elevated rod, and recorded each species that touched the string, and recorded abundance as

TABLE 1 List of plant species used to assess root fungal endophytes

Species	Abbreviation	Family	Status	Sample size
Agrostis gigantea	AGGI	Poaceae	Exotic	6
Bromus inermis	BRIN	Poaceae	Exotic	6
Poa compressa	POCO	Poaceae	Exotic	5
Poa pratensis	POPR	Poaceae	Exotic	3
Hypericum perforatum	HYPE	Hypericaceae	Exotic	6
Vicia cracca	VICR	Fabaceae	Exotic	5
Fragaria virginiana	FRVI	Rosaceae	Native	6
Potentilla recta	PORE	Rosaceae	Exotic	3
Asclepias syriaca	ASSY	Asclepiadoideae	Native	6
Convolvulus arvensis	COAR	Convolvulaceae	Exotic	6
Prunella vulgaris	PRVU	Lamiaceae	Native	6
Plantago lanceolata	PLLA	Plantaginaceae	Exotic	5
Veronica officinalis	VEOF	Plantaginaceae	Exotic	5
Linaria vulgaris	LIVU	Plantaginaceae	Exotic	6
Daucus carota	DACA	Apiaceae	Exotic	5
Euthamia graminifolia	EUGR	Asteraceae	Native	6
Hieracium caespitosum	HICA	Asteraceae	Exotic	5
Symphyotrichum novae-angliae	SYNO	Asteraceae	Native	6
Taraxacum officinale	TAOF	Asteraceae	Native	5
Centaurea jacea	CEJA	Asteraceae	Native	5
Cirsium arvense	CIAR	Asteraceae	Exotic	6
Tragopogon pratensis	TRPR	Asteraceae	Exotic	6
Rudbeckia serotina	RUSE	Asteraceae	Native	5
Leucanthemum vulgare	LEVU	Asteraceae	Exotic	6
Achillea millefolium	ACMI	Asteraceae	Native	4
Solidago canadensis	SOCA	Asteraceae	Native	4
Erigeron strigosus	ERST	Asteraceae	Native	5

the number of touches for each species. Plots were surveyed in a random order from June 13 to 20, 2016.

2.3 | Whole plant sample collection and processing

Whole plant samples, including above- and below-ground specimens, were collected over the course of three days between July 11 and 13, 2016. All the targeted plant species were mature and few showed signs of senescence. Collected plant species were diverse in phylogeny, including 10 native and 17 exotic species (Table 1) and we collected six individual plants per species. To avoid spatial autocorrelation, replicate plant samples for each species were as spatially distant within the sampling site as possible and the sampling locations of each individual plant were recorded. We note that a few plant species (e.g. *Potentilla recta* and *Rudbeckia serotine*) were found within relatively confined areas (Figure S1). However, most of the plant species were collected from locations spread out within the grid (Figure S1), thus spatial autocorrelation was less likely in analyses of grouped data with plant species as a predictive variable. Plant samples were kept on ice in coolers and brought to Algoma University, Sault Ste. Marie, Ontario, Canada), where they were stored at 4°C until they were further processed within 6 days since the harvest.

Plant samples were processed in the same manner as described by Reininger, Martinez-Garcia, Sanderson, and Antunes (2015). Briefly, root systems were gently washed with cold tap water to remove attached soils and roots from other plants. From each individual plant, up to 1 g of finest roots (<2 mm in diameter) were excised, and surface sterilized in 30% hydrogen peroxide for 30 s. The reaction was stopped by placing the roots in 70% ethanol, and the roots were rinsed with PCR-grade water. The sterilized root samples were frozen at −80°C before being lyophilized (Labconco FreeZone 2.5, Kansas City, MO, USA), and stored at −20°C.

2.4 | DNA extraction, PCR and Illumina sequencing

Each root sample was ground in a 2-ml centrifuge tube with a tungsten carbide bead (3 mm diameter), using a shaker (Retsch MM 400, Newtown, PA, USA) for 2 min at 20 Hz following a protocol suggested by DNeasy 96 Plant Kit (Qiagen, Toronto, ON, Canada). Genomic DNA was extracted from a 20-mg sub-sample using DNeasy 96 Plant Kit (Qiagen). The extracted DNA was quantified using a Biotek™ Microplate Spectrophotometer (Winooski, VT, USA). The fungal ITS-2 region was amplified, using 5.8S-FUN (AACTTTYRRCAAYGGATCWCT) and ITS4-FUN (AGCCTCCGCTTATTGATATGCTTAART) primers (Taylor et al., 2016) modified for the Illumina MiSeq sequencing platform by fusing CS1 and CS2 linkers (22 base-pair sequences to connect the region of interest with additional sequences, including barcodes, required to Illumina sequencing), respectively.

Polymerase chain reactions were performed on a thermocycler (Mastercycler™ Pro, Eppendorf, Hamburg, Germany) using 50 µl assays, consisting of 25 µl of GoTaq Colourless Master Mix (Promega, Madison, WI, USA), 7.5 µl of BSA (5 ng/µl), 2.5 µl of each primer (10 µM), 15 µl of PCR-grade water, and 12.5 µl of a diluted genomic DNA template (5 ng/µl) in a 200-µl PCR tube for each sample. The thermal profile consisted of an initial denaturation and enzyme activation step of 96°C for 2 min, followed by 36 cycles of 94°C for 30 s, 58°C for 40 s and 72°C for 120 s, with a final extension of 72°C for 10 min. The quality of PCR products, including sequence lengths, was evaluated by agarose gel electrophoresis. Additional rounds of PCR were performed to fuse CS1/CS2 linker primers to the indices and adapters before Illumina MiSeq sequencing at Génome Québec (Montréal, QC, Canada). This process was performed on a thermocycler (Mastercycler™ Pro S, Eppendorf, Hamburg, Germany) using 20 µl assays, consisting of 2 µl of FastStart™ PCR Buffer (Roche Diagnostics, Laval, QC, Canada), 0.1 µl of FastStart™ High Fidelity Enzyme Blend (Roche Diagnostics), 1.44 µl of $MgCl_2$ (25 mM), 1.0 µl of DMSO (5% final concentration), 0.4 µl of KAPA dNTP Mix (10 mM, KAPA, Wilmington, MA, USA), 12.06 µl of PCR-grade water, 2 µl of index primers (2 µM), and 1 µl of 1st PCR amplicon (approx. 0.15 ng/µl) in a 200-µl PCR tube for each sample. The thermal profile consisted of an initial denaturation and enzyme activation step at 95°C for 10 min, followed by 15 cycles at 95°C for 15 s, 60°C for 30 s and 72°C for 60 s, with a final extension at 72°C for 10 min.

All the samples were sequenced in one Illumina MiSeq run to avoid run-to-run variation (Wen et al., 2017). Original sequences were deposited at GenBank of the National Center for Biotechnology Information (accession numbers: SAMN09573584 to SAMN09573725).

2.5 | Sequence data processing and bioinformatics

The Illumina sequences were processed in QIIME 1.9.0 toolkit (Caporaso et al., 2010) following Taylor et al. (2016). Briefly, chimeric sequences were identified using the abundance-based method via USEARCH (Edgar, 2010) for each sample and removed for downstream analyses. After assembling all the sequences, operational taxonomic units (OTUs) and their representative sequences were determined at the ≥97% similarity level of the nucleotide sequences (Stackebrandt & Goebel, 1994). From each of the representative sequences, ITS2 sequences were extracted using ITSx (Bengtsson-Palme et al., 2013). Using the ITS2 sequences, taxonomy was assigned to each OTU by BLAST (Altschul, Gish, Miller, Myers, & Lipman, 1990) against the UNITE database (Kõljalg et al., 2013). OTUs with no BLAST hit, which mostly originated from host plants (Figure S2), were removed, resulting in varying sequence numbers ranging from 1,323 to 79,792 sequences per sample. Individual samples with less than 5,695 sequences were removed for downstream analyses, resulting in a total of 142 samples (Table 1). The resulting sequences were rarefied at 5,695 sequences per sample, and alpha diversity indices of the whole fungal communities for each root sample were calculated in QIIME, including Chao1 (Chao, 1984), observed OTUs and Shannon Index (Ludwig & Reynolds, 1988). The rarefied dataset contained a total of 3,186 fungal OTUs. Of the 3,186 OTUs, 868 OTUs were singletons, which were removed for downstream beta diversity

analyses because they comprised only 0.1% of the total sequences. This resulted in a total of 807,867 sequences in 2,318 OTUs, ranging from 5,672 to 5,695 sequences per sample. This resulting difference in sequencing depths was accounted for as a co-variate in all the subsequent beta diversity analyses of fungal communities. FUNGuild (Nguyen et al., 2016) was used to categorize the OTUs into functional groups. FUNGuild (Nguyen et al., 2016) is a database which annotates fungal OTUs into different ecological guilds, such as plant antagonists (e.g. plant pathogens), mutualists (e.g. mycorrhizal fungi and endophytes) and others (e.g. saprotrophs) with varying degrees of certainty based on literature. In this study, our selected functional groups of interest were AM fungi ('Arbuscular Mycorrhizal' in 'Guild' with 'Highly Probable' in 'Confidence Ranking') and plant pathogens ('Plant Pathogen' in 'Guild' with 'Probable' in 'Confidence Ranking'), which could influence plant community dynamics (van der Heijden et al., 1998; Mills & Bever, 1998). Of the 2,318 OTUs, 525 and 65 were categorized as AM fungi and plant pathogens, respectively. Resulting OTU tables from FUNGuild were used to assess beta diversity of AM and pathogenic fungi without rarefaction.

2.6 | Statistical analyses

All the computation in data analyses was conducted in R 3.4.2 (R Core Team, 2017). A phylogenetic tree of the host plants was constructed via Phylomatic (Webb & Donoghue, 2005), using the angiosperm phylogeny group reference tree (APGIII, R20120829), with branch lengths in millions of years derived from Wikström Savolainen and Chase (2001). A phylogenetic distance matrix was created by calculating pairwise distances among the host plants in the phylogenetic tree using the *cophenetic* function. A distance matrix for sampling locations of individual plant samples in the study site was also created using the *as.matrix* function.

To assess whether the plant species were a significant factor on the three alpha diversity indices of the fungal communities, we employed one-way ANOVAs. To assess differences in root fungal community structure among individual plant samples, non-metric multi-dimensional scaling (NMDS, Kruskal, 1964) was employed to calculate Bray–Curtis dissimilarity in two dimensions using the *vegan* package (Oksanen et al., 2007). The NMDS was implemented for the whole fungal community as well as communities of AM fungi and plant pathogens, which were assigned by FUNGuild (Nguyen et al., 2016). To achieve convergence in NMDS for AM fungi and plant pathogens, samples with low abundances were removed. Specifically, in NMDS for AM fungi, 16 samples with less than ten counts were removed, resulting in 126 samples, and in NMDS for pathogens, 24 samples with less than three were removed, resulting in 118 samples. We note that NMDS analyses for each fungal dataset were conducted using both two- and three-dimensional solutions. In two-dimensional NMDS solutions, stress values for the whole and AM fungal data were not set low enough for sound interpretations (i.e. >0.2, Clarke, 1993), compared with those in three-dimensional solutions (i.e. <0.2, Table S1). To compare the results of two- and three-dimensional NMDS, we employed Mantel tests (Mantel, 1967) which can assess the correlation between two matrices. The matrices created using the two- and three-dimensional NMDS solutions for each dataset were highly correlated (R = 0.920, 0.932 and 0.942 for whole, AM and pathogenic fungal datasets, respectively, in Mantel tests), and Mantel tests using two- and three-dimensional NMDS solutions to assess the relationship between fungal community structure and two predictive variables (i.e. host plant phylogeny and spatial arrangements of individual host plants) provided very similar results, thus leading to the same conclusions (Table S2). Therefore, despite the higher stress values, results of two-dimensional NMDS solutions are presented here due to technical difficulties in presenting visually interpretable results of three-dimensional NMDS solutions.

To evaluate contributions of the host plant phylogeny and sampling locations of individual plants on root-inhabiting fungal community structure, Mantel tests were conducted using the *vegan* package (Oksanen et al., 2007). To assess the correlation between plant phylogeny and fungal community structure, Mantel tests were performed using NMDS scores averaged by plant species, as well as NMDS scores of individual samples. First, pairwise distance matrices were calculated for the whole, AM and pathogenic fungal communities using mean as well as individual NMDS scores. Next, a pairwise distance matrix for individual plant samples and a pairwise phylogenetic distance matrix were constructed as previously described. Finally, the resulting matrices were used to perform Mantel tests to assess correlations between the root fungal community structure and host plant phylogeny as well as sampling locations of individual plants. We note that the phylogenetic distance within each host species is zero in correlation analyses using individual NMDS scores in Mantel tests. Therefore, the correlation results with individual NMDS scores would reflect host plant specificity of fungal community structure (i.e. similarity within each host plant species), if any, in addition to the relationship between plant phylogeny and fungal community structure.

To test if plant species had distinct root-inhabiting fungal communities, a permutational multivariate ANOVA (PERMANOVA) was conducted for each fungal group, using the *adonis* function in the *vegan* package (Oksanen et al., 2007). In each analysis, sequencing depths were included in the model as a covariate to account for their potential contributions. When the interaction effect between plant species and sequencing depths was not significant, only the main effects of plant species and sequencing depths were included in the model. To assess relative contributions of plant species and sequencing depths, R^2 and p-values were obtained with 999 permutations.

To determine if the relative abundances of AM and pathogenic fungi were specific to host plant species, Kruskal–Wallis tests (Kruskal & Wallis, 1952) were conducted in R 3.4.2. In addition, a differential abundance test was conducted using an analysis of composition of microbiomes (ANCOM, Mandal et al., 2015). Counts of OTUs categorized as AM fungi and plant pathogens via FUNGuild were binned for the respective fungal groups in the OTU table without singletons described above. In the analysis, using ANCOM-II (Kaul, Mandal, Davidov, & Peddada, 2017) in R 3.4.2., plant species and sequencing depths were specified as the main predictive variable and covariate, respectively.

Only OTUs with a proportion of zero counts smaller than 75% were assessed to exclude sparsely observed OTUs, and 0.05 in α-level was employed as the level of significance. To determine if the relative abundances of AM and pathogenic fungi in their roots were different due to the plants' geographic origins (i.e. native vs. exotic), one-way ANOVAs with 'origin' as a predictive variable were performed, using values averaged by plant species. In performing ANOVAs, appropriate data transformation was applied to response variables, when necessary, to meet assumptions of normality and equal variances. To determine if these relative abundances were influenced by plant phylogeny, Pagel's Lambda (λ) was calculated (Freckleton, Harvey, & Pagel, 2002; Pagel, 1999) and *K*-statistics were conducted (Blomberg, Garland, & Ives, 2003).

To detect fungal families whose relative abundances were significantly correlated with the observed host plant abundances, a differential abundance test using ANCOM was first implemented. The goal was to find fungal families whose relative abundances were significantly different among the host plants in a similar manner as described above. For fungal families whose relative abundances were significantly different among the host plant species, their average and median values of relative abundances and host plant abundances were assessed for Pearson's correlations.

3 | RESULTS

3.1 | Community structure and diversity of root-inhabiting fungi in an old field

Overall, Ascomycota dominated the fungal community, followed by Glomeromycota and Basidiomycota (Figure 1). There was, however, considerable variation in fungal diversity among the 27 plant species. Relative abundances of Ascomycota ranged from 46.6% (*S. novae-angliae*) to 97.7% (*L. vulgaris*), Glomeromycota from 0.1% (*P. pratensis*) to 51.8% (*S. novae-angliae*), and Basidiomycota from 0.7% (*E. graminifolia*) to 24.4% (FRVI) (Figure 1). Among classes belonging to the phylum Ascomycota, Leotiomycetes was the most dominant (overall mean 43.0%), followed by Dothideomycetes (overall mean 20.0%), Sordariomycetes (overall mean 9.8%), and Eurotiomycetes (overall mean 1.8%). Glomeromycetes was the most dominant class in the phylum Glomeromycota, with an overall mean of 5.9%, ranging from 0.1% (*P. pratensis*) to 44.9% (*S. novae-angliae*) (Figure 1). Agaricomycetes was the most dominant class belonging to phylum Basidiomycota, ranging from 0.3% (*A. syriaca*) to 24.0% (*F. virginiana*) (Figure 1).

3.2 | Alpha diversity of root-inhabiting fungal communities

Three alpha diversity indices, Chao 1, numbers of OTUs and Shannon index, were quantified for communities of root-inhabiting fungi (Figure 2). All three indices were, to some extent, controlled by host plant species, as suggested by the significant plant species effects for Chao 1 ($F_{26,115} = 3.463$, $p < 0.001$), observed number of fungal OTUs ($F_{26,115} = 3.625$, $p < 0.001$) and Shannon indices ($F_{26,115} = 2.627$, $p < 0.001$) in one-way ANOVAs. Chao1 and observed OTUs were influenced by host plant phylogeny, suggested by significant results of *K* statistics (Figure 2). No significant signal of host plant phylogeny was found for Shannon indices (Figure 2). When these alpha diversity indices were compared for host plant origins, no significant differences were detected via Student's *t* tests (Figure 2).

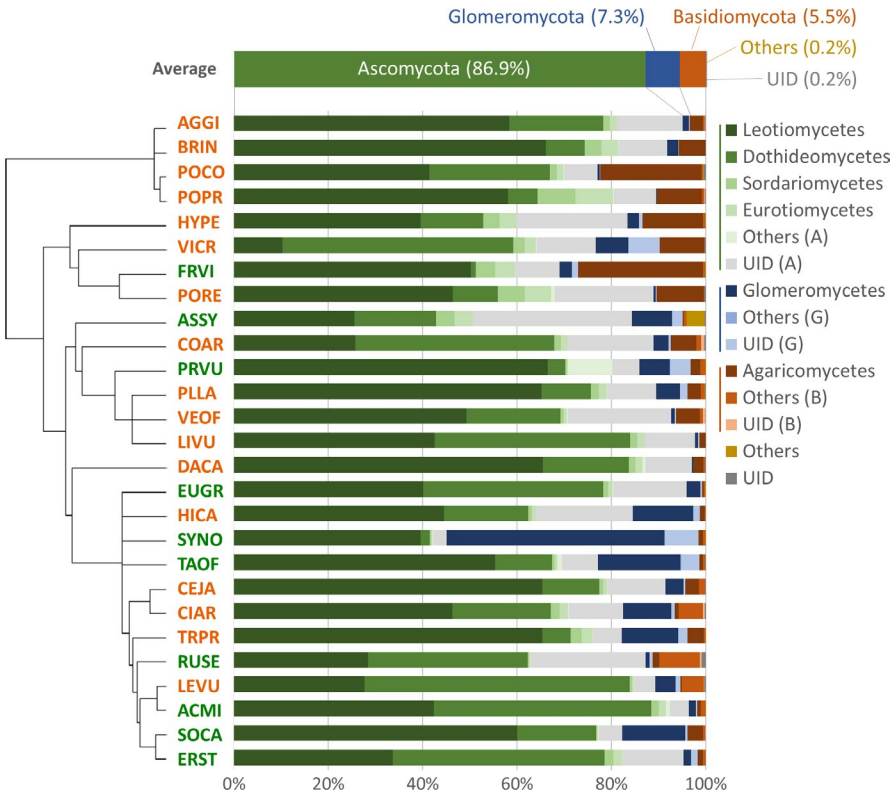

FIGURE 1 Mean relative abundances of root fungal endophytes at the class level in the 27 plant species. Species abbreviations (Table 1) in green and orange indicate native and exotic species, respectively. Others (A), (G) and (B) in the legend represent OTUs in classes of low relative abundance in phylum Ascomycota, Glomeromycota and Basidiomycota, respectively. UID (A), (G) and (B) in the legend represent unidentified OTUs at the class level belonging to phylum Ascomycota, Glomeromycota and Basidiomycota, respectively. Others represent OTUs in phyla of low relative abundance other than the three major phyla. UID represents OTUs unidentified at the phylum level [Colour figure can be viewed at wileyonlinelibrary.com]

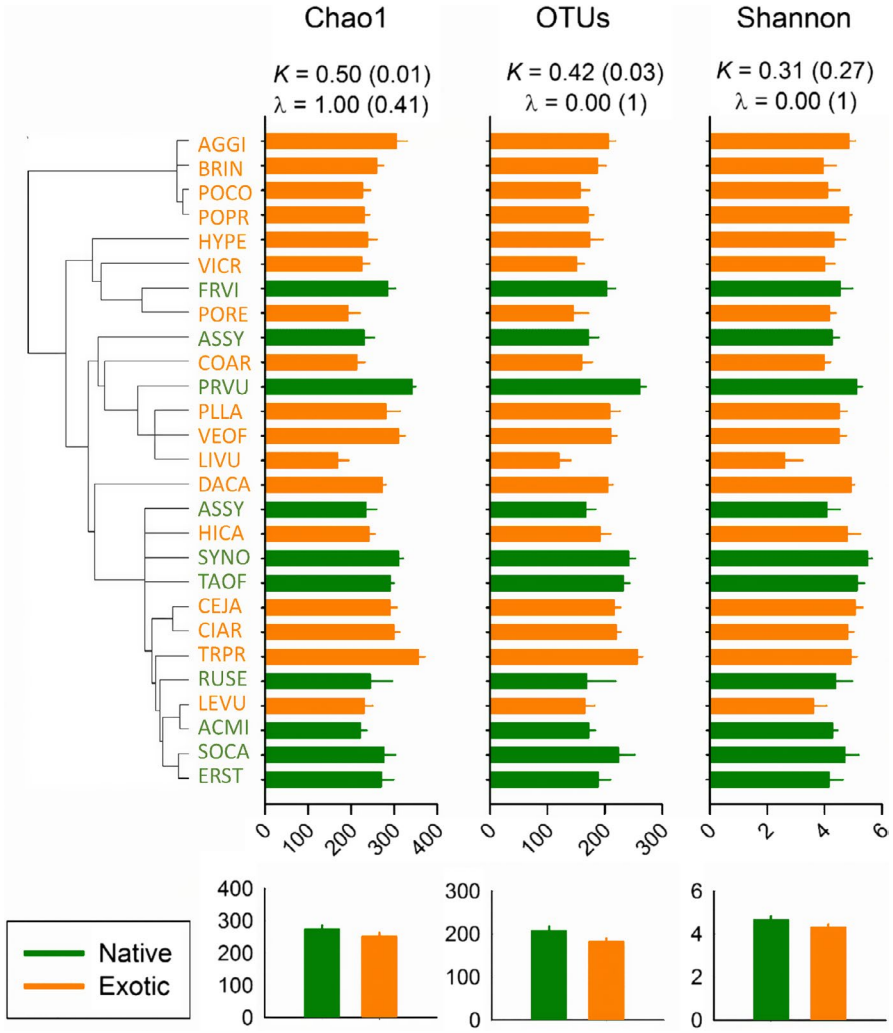

FIGURE 2 Alpha diversity of root fungal endophytes in the 27 plant species assessed using the dataset rarefied at 5,695 sequences per sample (top). *K* and λ (*p*-values between brackets) associated with each bar graph are results of analyses to detect phylogenetic signals of host plants. Alpha diversity values were compared regarding their origins (i.e. native vs. exotic, bottom) where no significant differences were found. Green and orange colours indicate native and exotic plants, respectively. [Colour figure can be viewed at wileyonlinelibrary.com]

3.3 | Beta diversity of root-inhabiting fungal communities

Community structure of the whole root-inhabiting fungi was specific to host plant species (Figure 3a). This was supported by significant plant species effects via PERMANOVAs for all plants as well as for native and exotic plants when accounting sequencing depths (Table 2). The structure of AM fungal communities (Figure 3c) depended on both host plant species and sequencing depths, as supported by significant interaction effects for all plants as well as for native and exotic plants (Table 2). The same trends were found for pathogenic fungal communities (Figure 3e), which was supported by significant interaction effects between plant species and sequencing depths (Table 2).

The community structure of all fungi was primarily shaped by host plant phylogeny, as determined by NMDS and Mantel tests (Figure 3). When NMDS scores averaged by host plant species were used, host plant phylogeny was a significant predictor for fungal community structure for all plants and for exotic plants (Figure 3). For native plants, a significant correlation was found only when using individual NMDS scores (Figure 3), reflecting high host specificity. Using all the plant species excluding the four grass species, however, the phylogenetic signal of host plants in mean NMDS scores was lost ($R = 0.11$, $p = 0.165$ via a Mantel test). Using all the exotic plant species excluding the four grass species, a phylogenetic signal of host plants was only marginally significant in the mean NMDS scores ($R = 0.33$, $p = 0.067$ via a Mantel test). Spatial locations of individual host plants did not appear to influence the overall fungal community structure of either all, native or exotic plants (Figure 3).

When focusing on AM fungal community structure, the spatial arrangements of individual host plants were an important predictor, irrespective of host plant origin (Figure 3). However, no significant correlation was found between host plant phylogeny and community structure of AM fungi when NMDS scores were averaged by host species (Figure 3). A significant correlation was found only when individual NMDS scores were used in a Mantel test for all plants, reflecting high host specificity.

Host plant phylogeny and spatial locations of individual plants appeared to determine fungal pathogenic community structure in roots (Figure 3). Host plant phylogeny was a significant predictor of community structure of pathogenic fungi for all plant species and exotic plants when NMDS scores were averaged by host species (Figure 3). For native plants, plant phylogeny was a significant predictor only when individual NMDS scores were used, reflecting high

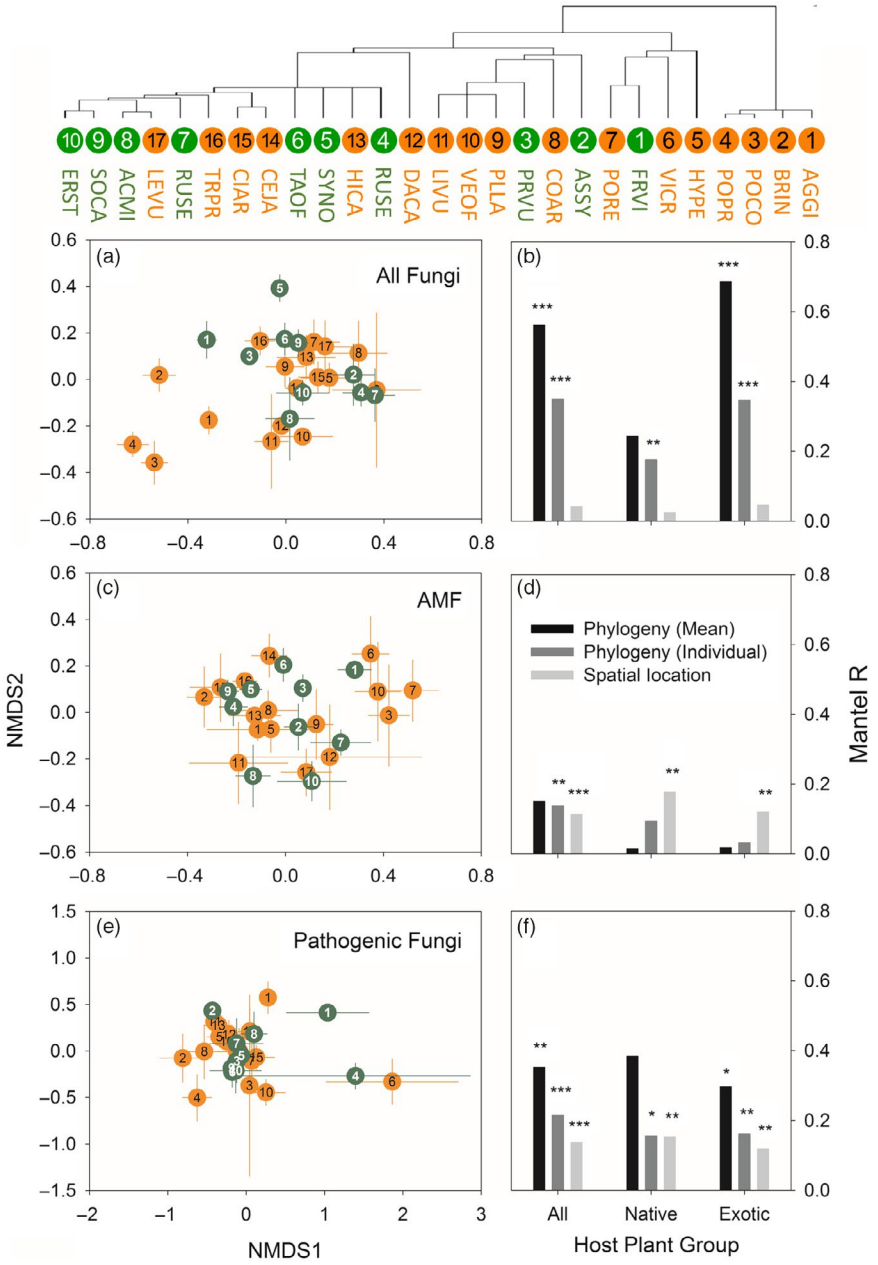

FIGURE 3 Results of NMDS for community structure of all, AM, and pathogenic fungi (a, c and e, respectively) and corresponding Mantel tests to assess correlation between distance matrices from NMDS scores and two predictive variables (i.e. distance matrices calculated from host plant phylogeny and sampling locations of individual host plants) (b, d and f). Abbreviations of plant species are listed in Table 1. Green and orange colours indicate native and exotic plant species, respectively. Colours and numbers of symbols in NMDS plots correspond to plant species associated with symbols under the phylogenetic tree. Error bars represent standard errors. Symbols above bars in the right panels indicate statistical significance in Mantel tests; * <0.05, ** <0.01, and *** <0.001. Note: *R* values from Mantel tests on right panels may not be directly comparable across host plant groups and predictive variables because of different sample sizes [Colour figure can be viewed at wileyonlinelibrary.com]

host specificity of pathogenic community structure. Spatial locations of host plants were significant predictors for community structure of pathogenic fungi, regardless of host plant origins (Figure 3).

3.4 | Relative abundances of host plants and of their root-inhabiting pathogenic and AM fungi

Relative abundances of the 27 plant species varied, with *Poa* spp being the most abundant and *T. officinale* the lowest (Figure 4). Two plant species, *H. perforatum* and *A. syriaca*, were only found in-between the permanent plots. No significant signal of plant phylogeny was found in plant abundances (Figure 4). There was also no significant difference in the overall abundance between native and exotic plant species (Figure 4).

Relative abundances of AM and pathogenic fungi varied among the 27 plant species (Figure 4). The abundances were plant species specific as supported by Kruskal–Wallis tests where plant species effects on the relative abundances of AM fungi (χ^2 = 71.5, *df* = 26, $p < 0.001$) and pathogens (χ^2 = 47.0, *df* = 26, $p = 0.007$) were significant. Host specificity based on the relative abundances of AM fungi was also supported by ANCOM (W = 160 out of 164, or 97.6%, Table S3). On the other hand, ANCOM indicated that the host specificity based on the relative abundances of pathogens was relatively weak (W = 123 out of 164, or 75.0%, Table S3). Relative AM fungal abundances appeared controlled by the phylogeny of their host plants, as suggested by a significant Pagel's λ ($p = 0.02$) and a marginally significant result of *K*-statistics ($K = 0.54$, $p = 0.09$) (Figure 4). When values averaged by species were compared regarding their host

TABLE 2 Results of PERMANOVAs to assess relative contributions of host plant species and sequencing depths on community structure of all, AM and pathogenic fungi

Fungal group	Host plant category	Host plant spp	Sequencing depths	Interaction
All fungi	All	R^2 = 0.36 p = 0.001	R^2 = 0.01 p = 0.001	N/A
	Native	R^2 = 0.36 p = 0.001	R^2 = 0.02 p = 0.09	N/A
	Exotic	R^2 = 0.34 p = 0.001	R^2 = 0.01 p = 0.026	N/A
AM fungi	All	R^2 = 0.30 p = 0.001	R^2 = 0.03 p = 0.001	R^2 = 0.20 p = 0.001
	Native	R^2 = 0.23 p = 0.001	R^2 = 0.05 p = 0.001	R^2 = 0.19 p = 0.001
	Exotic	R^2 = 0.26 p = 0.001	R^2 = 0.03 p = 0.001	R^2 = 0.20 p = 0.041
Pathogens	All	R^2 = 0.31 p = 0.001	R^2 = 0.03 p = 0.001	R^2 = 0.27 p = 0.001
	Native	R^2 = 0.26 p = 0.006	R^2 = 0.07 p = 0.001	R^2 = 0.24 p = 0.015
	Exotic	R^2 = 0.32 p = 0.001	R^2 = 0.03 p = 0.001	R^2 = 0.26 p = 0.001

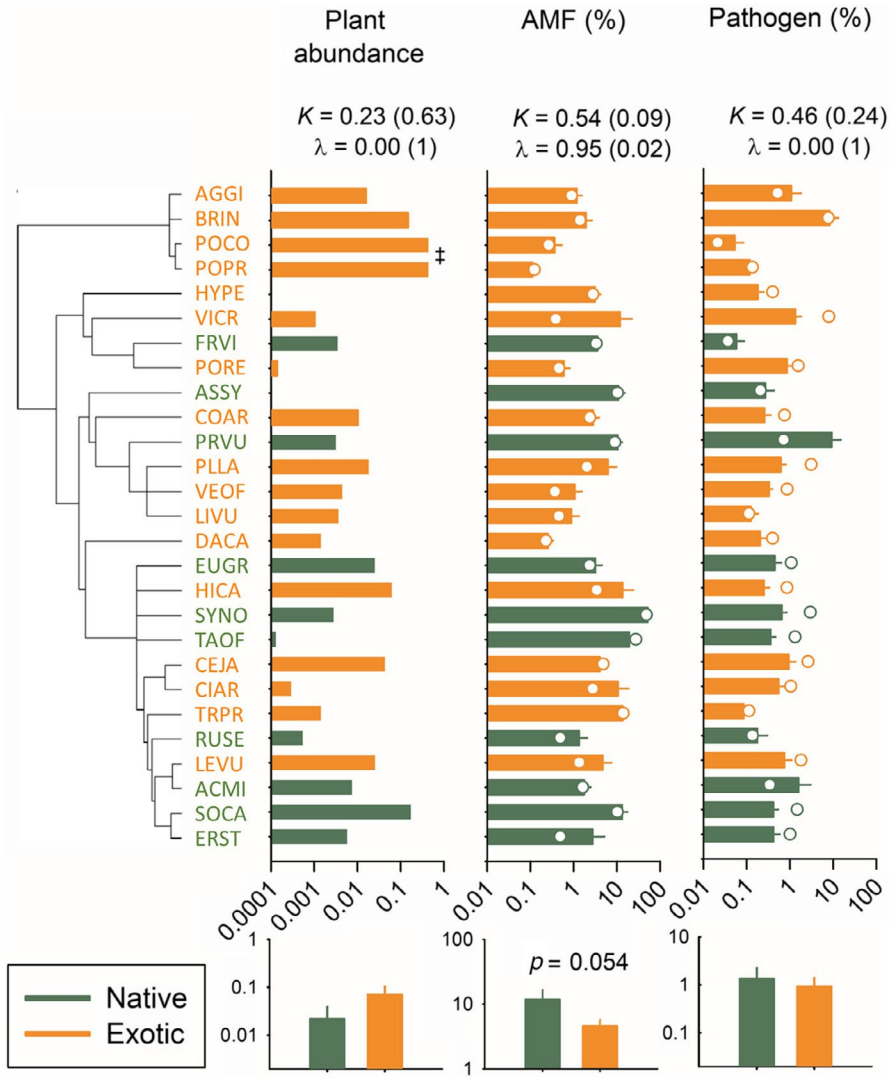

FIGURE 4 Relative abundances of host plant species in the study site, and average relative abundances of AM and pathogenic fungi in roots of host plants (top). White circles show median values. K and λ (p-values between brackets) associated with each bar graph are results of analyses to detect phylogenetic signals of the host plants using average values. Measurements in each upper graph were compared regarding origins of host plants (i.e. native vs. exotic, bottom). Relative AM fungal abundances were different between native and exotic host plants with marginal significance. ‡POCO and POPR could not be distinguished when plant relative abundances were assessed in June 2016. Green and orange colours indicate native and exotic plants, respectively. [Colour figure can be viewed at wileyonlinelibrary.com]

plants' origins (native vs. exotic), marginally significant differences were found in the relative abundances of AM fungi ($F_{1,25}$ = 4.09, p = 0.054), where AM fungal abundances were higher in native (11.9%) than in exotic plant species (4.6%) (Figure 4). When the four exotic grass species were excluded, however, the marginally significant difference between the native and exotic species was lost ($F_{1,21}$ = 1.74, p = 0.201, via ANOVA).

3.5 | Correlation between community structure of root-inhabiting fungi and the relative abundances of host plants

Relative abundances of plant pathogens and AM fungi were not significant predictors of the observed plant abundances (Figure 5, Table 3). This was also the case when correlation analyses were conducted for native and exotic plant species, separately (Table 3).

Among the 27 root-inhabiting fungal families detected in this study, six families (Cladosporiaceae, Glomeraceae, Nectriaceae, Pleosporaceae, Magnaporthaceae and Leotiaceae) had distinct relative abundances among their host plants (Table S4). When relative abundances of the six families were assessed for Pearson's correlations with their host plant abundances, Cladosporiaceae and Magnaporthaceae had significant relationships (Table 3). Specifically, the average relative abundances of Cladosporiaceae were negatively correlated with their host plant abundances when all the plant species were considered (Table 3, Figure 5). Median relative abundances of Magnaporthaceae were positively correlated with their host plant species, which was driven by exotic plants (Table 3, Figure 5).

4 | DISCUSSION

To our knowledge, this is the first study to document a significant relationship between community structure of root-inhabiting fungi and phylogeny of their diverse host plants, including natives as well as exotics, using high-throughput sequencing. Communities of AM

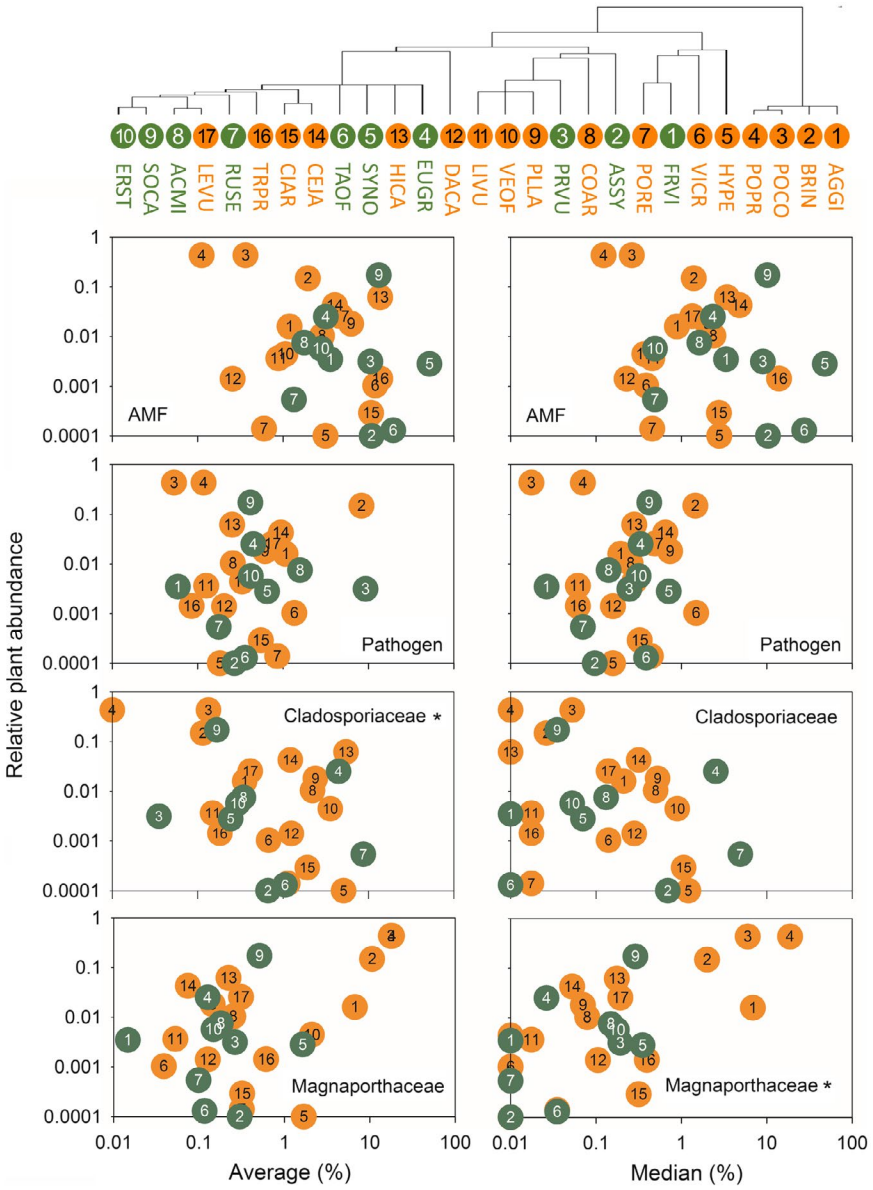

FIGURE 5 Relative abundances of the 27 plants are plotted against relative abundances (averages and medians) of two fungal groups (AM and pathogenic fungi) and two fungal families (Cladosporiaceae and Magnaporthaceae). Results of Pearson's correlation analyses are shown in Table 3. Asterisks next to fungal family names indicate significant correlation (Table 3). Green and orange colours indicate native and exotic plants, respectively. Colours and numbers of symbols in each plot correspond to plant species associated with symbols under the phylogenetic tree [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Results of Pearson's correlation analyses between relative abundance (averages and medians) of selected fungal groups and relative plant abundance. The selected fungal groups include AM and pathogenic fungi, and six fungal families whose relative abundances were specific to host plant species

Fungal group	Values	All plants	Native	Exotic
AMF[a]	Average	p = 0.185 R = −0.26	p = 0.66 R = −0.16	p = 0.331 R = −0.24
	Median	p = 0.185 R = −0.26	p = 0.585 R = −0.20	p = 0.400 R = −0.22
Pathogens[a]	Average	p = 0.669 R = 0.09	p = 0.602 R = 0.19	p = 0.859 R = 0.05
	Median	p = 0.794 R = 0.05	p = 0.384 R = 0.31	p = 0.718 R = −0.09
Cladosporiaceae[a]	Average	**p = 0.050** **R = −0.38**	p = 0.572 R = −0.20	p = 0.060 R = −0.46
	Median	p = 0.240 R = −0.22	p = 0.926 R = 0.03	p = 3.90 R = −0.45
Glomeraceae	Average	p = 0.288 R = −0.21	p = 0.778 R = −0.10	p = 0.454 R = −0.19
	Median	p = 0.242 R = −0.23	p = 0.697 R = −0.14	p = 0.438 R = −0.20
Nectriaceae	Average	p = 0.213 R = −0.25	p = 0.870 R = −0.06	p = 0.138 R = −0.37
	Median	p = 0.744 R = −0.07	p = 0.991 R < 0.01	p = 0.125 R = −0.39
Pleosporaceae	Average	p = 0.415 R = −0.16	p = 0.700 R = −0.14	p = 0.161 R = −0.36
	Median	p = 0.461 R = −0.15	p = 0.797 R = 0.09	p = 0.076 R = −0.44
Magnaporthaceae[a]	Average	p = 0.146 R = 0.29	p = 0.928 R = 0.033	p = 0.195 R = 0.33
	Median	**p < 0.001** **R = 0.63**	p = 0.074 R = 0.59	**p = 0.006** **R = 0.64**
Leotiaceae	Average	p = 0.097 R = 0.33	p = 0.180 R = 0.46	p = 0.522 R = 0.17
	Median	p = 0.796 R = 0.05	p = 0.793 R = 0.10	p = 0.813 R = −0.06

Note. p-values ≤0.05 and associated R values are shown in bold.
[a]Correlations are shown in Figure 5.

fungi were structured by the spatial arrangements of host plants, but unlike the whole fungal community, were not associated with plant phylogeny. We demonstrate that both host plant phylogeny and spatial arrangements of individual host plants in the field were important predictors of the pathogenic fungal community structure. However, we found that the relative abundances of AM and pathogenic fungi quantified once during the growing season were not correlated with host plant abundances in the old field. This finding contradicts expectations derived from the known influence of root-inhabiting fungi on plant performance and competitive interactions, but nonetheless suggests that static root fungal community structure may not be tightly coupled with the composition of the plant community.

Our results indicated that there was a significant relationship between host plant phylogeny and the structure of root-inhabiting fungi which was driven primarily by exotic plant species in the old-field community, and particularly by the exotic grass species (Figure 3). This result is consistent with the finding by Wehner et al. (2014) using data from 25 plant species of Asteraceae in a semi-arid grassland. Similar relationships have been also reported in Orchids (across Europe, Jacquemyn et al., 2011) and Salicaceae species (across Estonia, Tedersoo et al., 2013) and their respective mycorrhizal fungal communities. Closely related plant species can share similar phenology (Davies et al., 2013) and root traits (Kembel & Cahill, 2005, 2011), which can facilitate colonization by root-inhabiting fungi, potentially explaining the phylogenetic signals between host plant and root-inhabiting fungal community structure (Wehner et al., 2014). The same mechanisms can be behind the observations that root-inhabiting bacteria are structured by host plant phylogeny (Fitzpatrick et al., 2018; Naylor, DeGraaf, Purdom, & Coleman-Derr, 2017; Yeoh et al., 2017). Functional similarity among closely related species may also explain the relationship between plant phylogenetic distance and fungal alpha-diversity indices found in this study (Figure 2).

As would be expected, the natural diversity of plant species in the old-field plant community was not phylogenetically balanced for native versus exotic plant species (Figure 3), and this affected our results. Fungal communities of the four grass species (*A. gigantean*, *B. inermis*, *P. compressa* and *P. pratensis*, Table 1), which were all exotic, created an independent and distant clade in the phylogenetic tree (Figure 3). This uniqueness of the four grass species contributed to a strong phylogenetic signal of host plants in community structure of root-inhabiting fungi when fungal communities of all plants were analysed (Figure 3). When the same analysis was conducted for all plants excluding the four grass species, the phylogenetic signal of host plants in mean NMDS scores was lost. In a similar analysis for exotic species excluding the four grass species, a phylogenetic signal of host plants was only marginally significant in the mean NMDS scores. Since similar studies are scarce, more research is necessary to test if grass species support distinct root-inhabiting fungal structure compared to forbs in a given community, as observed in this study.

We found evidence of host plant specificity for AM fungi which was supported by a significant plant species effect via a PERMANOVA of the similarity matrix (Figure 3c). Historically, AM fungi have been considered to possess low host plant specificity. This was due in part to the observation that any isolated AM fungus can associate with a wide range of plant species (see Smith and Read, 2008). However, using high-throughput sequencing, recent studies have demonstrated that host plant species can influence the structure of their AM fungal communities (Ciccolini et al., 2016; Davison et al., 2016; Vályi et al., 2015, but see Dumbrell et al., 2011). For example, though Öpik, Metsis, Daniell, Zobel, and Moora (2009) did not find significant host plant specificity in AM fungal community structure of 10 understory plant species in a temperate forest, the fungal communities differed between forest specialist and generalist species. Furthermore, experimental studies have established that certain combinations of AM fungal taxa and plant species can be preferably established due to reciprocal rewards between AM fungi and host plants (Kiers et al., 2011), and our findings suggest that these processes may influence the composition of root-inhabiting fungal communities. Nevertheless, this conclusion should be tempered for AM fungi in the NMDS analysis as differences in sampling depths among samples were also responsible for the effect of host plant identity, as suggested by significant interactions between host plant species and sequencing depths (Table 2).

Despite finding evidence for AM fungal specificity to host plant species, we did not find evidence for a phylogenetic signal of host plants on AM fungal community structure (Figure 3d). This finding is consistent with a synthesis study by Põlme et al. (2018) using previously published datasets. In a meta-analysis, Veresoglou and Rillig (2014) explored phylogenetic host specificity in AM fungal communities to reach a counter-intuitive conclusion that closely related co-occurring plants tend to have dissimilar AM fungal communities. This conclusion was supported by Reinhart and Anacker (2014) who reported a similar trend for 10 dominant plants and associated AM fungi in three grasslands. In this study, however, a Mantel test examining the correlation between NMDS scores of AM fungal communities and host plant phylogenetic distances showed a positive slope, indicating that closely related plant species tended to have more similar AM fungal communities, though the relationship was not statistically significant (Figure 3d).

The spatial arrangements of individual host plants predicted AM fungal community structure, regardless of host plant origins (Figure 3d). Though this result may appear to contradict that of high AM fungal host plant specificity (Figure 3c), it is likely that both the host plant identities and the spatial arrangement of individual host plants in the field contributed to structuring AM fungal communities in roots. However, their contributions could not be clearly teased apart because of potential spatial autocorrelations of some plant species sampled in any observational study (e.g. *P. recta* and *R. serotine*, Figure S1). Spatial distance can be a major factor in shaping AM fungal communities in roots in a similar spatial scale used in this study (Horn et al., 2017; Maherali & Klironomos, 2012), and dispersal limitation of AM fungi can be a dominant mechanism behind this observation (Vályi et al., 2016). This apparent dispersal inability can cause AM fungal community to structure at scales of <1 m (Mummey & Rillig, 2008).

We were interested in potential differences in fungal communities between the native and exotic plant species, especially in pathogenic fungi, in the context of the enemy release hypothesis (Inderjit & van der Putten, 2010; Mitchell & Power, 2003). However, we did not find distinct differences in fungal community characteristics between the native and exotic plant species, except for relative abundances of AM fungi (Figures 2–4). Some past studies explored differences in belowground plant microbiomes between the two groups, and the results were mixed; little differences were reported for dark septate endophytic fungi in roots (Knapp, Pintye, & Kovács, 2012), root-inhabiting fungi in dune grasses (Johansen et al., 2016), and rhizosphere fungal communities of *Acer* species (Toole et al., 2018) and *Alternanthera* species (Lu, He, Ding, & Siemann, 2018), whereas distinct differences were found in AM fungal communities associated with native and exotic forbs (Bunn, Lekberg, Gallagher, Rosendahl, & Ramsey, 2014). Given the similar trends between the two groups in the beta diversity analyses (Figure 3), the exotic plant species might have been naturalized in the region for so long (Table S5) that they established belowground plant–microbial interaction similar to that of the native plants (Diez et al., 2010). The higher relative abundances of AM fungi in native than the exotic species was, in part, driven by the four exotic grass species with relatively low AM fungal abundances (Figure 4). Without the four exotic grass species, no significant difference was detected between the native and exotic species. The lower relative abundances of AM fungi of the four cool-season C_3 grass species are consistent with other observations showing that, compared to other functional groups, this group of plants tends to have lower AM fungal colonization (Hoeksema et al., 2010; Reinhart, Wilson, & Rinella, 2012; Wilson & Hartnett, 1998).

Community structure of pathogenic fungi appeared to be shaped by both host plant phylogeny and the spatial locations of individual

host plants (Figure 3e,f). Host specificity of plant pathogens varies along a specialist-to-generalist continuum, and specialization of a pathogen can be gauged by phylogenetic breadth of host plants which the pathogen can infect (Barrett, Kniskern, Bodenhausen, Zhang, & Bergelson, 2009). As such, closely related plant species are more likely to be infected by a specialist pathogenic fungus (Gilbert & Webb, 2007; Parker et al., 2015). Thus, the effect of host plant phylogeny on the community structure of root pathogenic fungi (Figure 3) can indicate that a portion of pathogenic fungi present in roots were specialists which were shared closely related host plant species (e.g. Mills & Bever, 1998). The significant effects of spatial locations of host plants in the community structure of root pathogenic fungi (Figure 3) might reflect dispersal limitation of soil-borne pathogenic fungi. Soil-borne pathogenic fungi disperse through spores and hyphae, similar to AM fungi (Wallace, 2012), and various environmental conditions, such as tortuosity and continuity of air-filled pore space in soils (Otten & Gilligan, 1998; Otten, Gilligan, Watts, Dexter, & Hall, 1999) and bulk density (Harris, Young, Gilligan, Otten, & Ritz, 2003; Otten et al., 2001) and presence of vectors (e.g. earthworms, Doube, Stephens, Davoren, & Ryder, 1994) can affect their dispersal.

The effect of plant–microbe interactions, a potential driver of plant community composition (Anacker et al., 2014; Bennett & Cahill, 2016; Kempel, Rindisbacher, Fischer, & Allan, 2018; Klironomos, 2002; Mangan et al., 2010; Teste et al., 2017), is determined by the net result of negative and positive effects by pathogens and mutualists (e.g. AM fungi), respectively (Reinhart & Callaway, 2006). Though we did not find a clear association between fungal abundance and plant abundance, these above- and below-ground interactions are dynamic, changing both seasonally and from year to year (Brundrett & Kendrick, 1988; Davison, Öpik, Daniell, Moora, & Zobel, 2011; Dumbrell et al., 2011). As such, a one-time assessment of root pathogens and mutualists, employed in this study, may not be sufficient for predicting community composition of host plants in a field. Another possible explanation is that we assessed relative abundances of AM and pathogenic fungi via Illumina sequencing, but absolute abundances of these fungal groups might be more appropriate to test the predictions. It is also possible that the version of FUNGuild (Nguyen et al., 2016) used in this study might not capture all the pathogenic fungi. In this study, 38.5% of the OTUs (892 out of 2,318 OTUs) were unassigned to any guilds. However, as Nguyen et al. (2016) stated, ongoing efforts to expand the database are improving the capability of FUNGuild. Other non-fungal pathogens, such as bacteria and oomycetes (Philippot, Raaijmakers, Lemanceau, & Putten, 2013), might be another driver in shaping plant community composition. There could be more influential factors structuring the plant community in the old field than the interaction between root-inhabiting fungi and host plants. For instance, influences of plant traits (Craine, Froehle, Tilman, Wedin, & Chapin, 2001; Fynn, Morris, & Kirkman, 2005; Reader, 1998) and aboveground microbiome (Whitaker, Bauer, Bever, & Clay, 2017) could mask clear relationship between host plant abundances and their root-inhabiting fungal characteristics.

Fungal family Cladosporiaceae, whose relative abundances were negatively correlated with their host plant abundances, consisted of OTUs belonging to *Cladosporium delicatulum* in this study. In FUNGuild, *C. delicatulum* is categorized as belonging to multiple guilds, including plant pathogen (Amanelah Baharvandi & Zafari, 2015), endophyte (Materatski et al., 2018; Venkateswarulu et al., 2018) and wood saprotroph (Bensch, Braun, Groenewald, & Crous, 2012), indicating potential diverse ecological roles of *C. delicatulum* in plant roots. The significant negative correlation between the relative abundances of *C. delicatulum* and its host plant abundances (Figure 5) may suggest that *C. delicatulum* behaves as a root-inhabiting pathogen in this study site. Fungal family Magnaporthaceae, whose relative abundances were positively correlated with their host plant abundances, consisted in part of OTUs belonging to *Gaeumannomyces radicicola* in this study. In FUNGuild, *G. radicicola* is categorized as a plant pathogen, which appears inconsistent with the significant positive correlation between this fungus' relative abundances and their host plant abundances detected here (Figure 5, Table 3). However, pathogenic Magnaporthaceae are often associated with the roots of grass species (Luo, Walsh, & Zhang, 2015), which happened to be the most abundant group of host plants in our study site (Figure 4), providing high leverage points in the positive correlation (Figure 5). Such high abundance and host specificity of pathogenic Magnaporthaceae associated with highly abundant grass roots exemplifies the complex nature of roles that root-inhabiting fungi can play in shaping their host plant abundances.

In conclusion, factors that were most likely to structure root-inhabiting fungal communities depended on the taxonomic scale and functional group of fungi in an old-field plant community. Fungal community composition in general was influenced by host plant phylogeny but insensitive to spatial arrangements of host plants, whereas the opposite was the case for AM fungal communities, and pathogenic fungi appeared to be structured by both plant phylogeny and host plant spatial arrangement. The absence of distinct differences in root-inhabiting pathogenic fungal communities between the native and exotic plant species indicated that the exotic plant species are naturalized in the region having now established belowground plant–microbial interactions similar to those of native plants. Our findings have implications for the consequences of plant invasion on soil fungal communities. For example, the fact that host plant phylogeny plays a role in structuring the entire fungal community in roots, and particularly pathogenic fungi, suggests that changes in soil microbial communities following exotic plant introductions may be predicted by the phylogenetic affiliation of the host plant.

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AUTHORS' CONTRIBUTIONS

A.K. and P.M.A. conceived the ideas and designed methodology; A.K., P.M.A. and H.M. collected the data; A.K. performed the analyses and led the writing of the manuscript. All authors contributed to revising the manuscript and gave final approval for publication.

DATA ACCESSIBILITY

Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.46f1n9j (Koyama, Maherali, & Antunes, 2019).

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SUPPORTING INFORMATION

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

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