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Fungal community assembly in soils and roots under plant invasion and nitrogen deposition

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ABSTRACT

Fungal community composition in the Anthropocene is driven by rapid changes in environmental conditions caused by human activities. This study examines the relative importance of two global change drivers – atmospheric nitrogen (N) deposition and annual grass invasion – on structuring fungal communities in a California chaparral ecosystem, with emphasis on arbuscular mycorrhizal fungi. We used molecular markers, functional groupings, generalized linear statistics and joint distribution modeling, to examine how environmental variables structure taxonomic and functional composition of fungal communities. Invasive grasses had a lower richness and relative abundance of symbiotic fungi (both AMF and other fungi) compared to native shrubs. We found a higher richness and abundance of rhizophilic (e.g. Glomeraceae) and edaphophilic (e.g. Gigasporaceae) AMF with increasing soil NO_3. Our findings suggest that invasive persistence may decrease the presence of multiple soil symbionts that native species depend on for pathogen protection and increased access to soil resources.

1. Introduction

Soil fungal community composition responds strongly to drivers of global change such as non-native plant invasions and atmospheric nitrogen (N) deposition (Egerton-Warburton and Allen, 2000; Amend et al., 2015). The U.S. southwest is experiencing high rates of invasion from Mediterranean annual grasses facilitated by increased N deposition (Fenn et al., 2010; Ashbacher and Cleland, 2015). Decreases in plant diversity following invasion alter the composition and function of soil fungi via changes in litter inputs and symbiotic relationships (Wolfe and Klironomos, 2005; Reinhart and Callaway, 2006; Inderjit and van der Putten, 2010). N deposition is also altering fungal composition both directly through shifts in nutrient availability and indirectly via shifts in plant community composition. While vegetation responses to invasion and N deposition have been examined (Rao and Allen, 2010; Valliere et al., 2017), relatively little is known about soil fungal responses, despite recent efforts (Egerton-Warburton and Allen, 2000; Egerton-Warburton et al., 2001, 2007; Amend et al., 2015).

Many fungal functional groups may respond to drivers of global change, including arbuscular mycorrhizal fungi (AMF), ectomycorrhizal fungi (EMF), saprotrophs and pathogens. AMF are plant mutualists, providing host plants with resources (nutrients and water) in exchange for photosynthetically derived carbon. N deposition and invasion of non-native plant species have the potential to shift the structure and function of both AMF and broader fungal communities. N deposition can lead to soil eutrophication, which has the potential to reduce the dependence of host-plants on AMF for nutrient uptake (Treseder and Allen, 2002;

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Egerton-Warburton et al., 2007). Additionally, some invasive plants exhibit relatively low AMF dependence which could decrease the presence of AMF (Hawkes et al., 2006; Busby et al., 2011, 2013). Molecular advances have facilitated the discovery of substantial diversity within AMF. Yet, without determining the functional significance of specific AMF taxa, it is challenging to infer the ecological importance of shifts in taxa abundance (Peay, 2014).

The composition of AMF may be altered by invasive annual grasses from the Mediterranean that replace shrub communities (e.g. chaparral) in southern California (Egerton-Warburton and Allen, 2000). The mechanism for this shift in species composition may be related to host preference of AMF (Hausmann and Hawkes, 2009; Sikes et al., 2009), which could result in differences in community composition and function between invasive and native host plants. Fast-growing AMF taxa may preferentially colonize species with earlier root activity and more fibrous root structures that are well suited for rapid nutrient uptake, such as invasive grasses (Hooper and Vitousek, 1998). Increased presence of intraradical hyphae produced by these AMF taxa confer pathogen protection to vulnerable fibrous roots (Maherali and Klironomos, 2007; Sikes et al., 2010). Abundant fast-growing AMF taxa in the roots of invasive grasses may create a positive feedback loop and promote grass invasion. On the other hand, woody plant species such as native shrubs with slower growth rates and coarser root morphologies may be more dependent upon slower growing AMF taxa with their capacity for nutrient uptake via long extraradical hyphae (Hart and Reader, 2002; Allen et al., 2003; Maherali and Klironomos, 2007). Release from fungal pathogens could also promote the establishment of invasive plants (Mitchell and Power, 2003; Kardol et al., 2007; Van Grunsven et al., 2007; Reinhart et al., 2010), though pathogen release is less important in disturbed systems (Müller et al., 2016). In resource-poor environments where plants are heavily dependent on mycorrhizal relationships, disruptions of these mutualistic networks through invasion can promote the establishment and persistence of invasive plants (Richardson et al., 2000; Callaway et al., 2008; Busby et al., 2013).

AMF associations are not affected by their host plants alone, but also directly and indirectly by soil properties. Previous work has shown interactive effects of nitrogen (N) and phosphorus (P) on AMF taxa, such that in P rich soil (lower N:P ratio) nitrogen fertilization decreases AMF productivity and diversity (Treseder and Allen, 2002; Egerton-Warburton et al., 2007). At P-limited sites, fertilization often increases AMF productivity and diversity (Treseder and Allen, 2002; Egerton-Warburton et al., 2007). However, as nutrient availability increases, it is likely that host plants will depend less on AMF taxa that produce extraradical hyphae for nutrient uptake (Sikes et al., 2010). Invasion by exotic annual plants has been linked to the rise in N deposition in southern California (Rao and Allen, 2010; Valliere et al., 2017). Therefore, invasion and N deposition may synergistically decrease the diversity and abundance of slower growing AMF families.

AMF have been previously placed into functional groups as early and late successional by spore size (e.g. Allen et al., 2003). Alternatively, the guild approach outlined in Weber et al. (2019, this issue), organizes AMF families by patterns of biomass allocation (Table 1), synthesized from previous studies (Hart and Reader, 2002; Powell et al., 2009; Varela-Cervero et al. 2015, 2016a, 2016b). Briefly, this approach classifies AMF families with high allocation to extradical hyphae as 'edaphophilic,' those with high allocation to root colonization as 'rhizophilic,' and those with lower allocation to either root colonization or soil hyphae than the edaphophilic or rhizophilic guilds as 'ancestral.' Families in the edaphophilic guild improve plant nutrient uptake, whereas families in the rhizophilic guild may protect host plant roots from pathogen colonization (Sikes et al., 2010; Treseder et al., 2018).

Table 1
Description of AMF Functional Groups adapted from (Weber et al., 2019).

Functional Group	Intraradical Hyphae	Extraradical Hyphae	Families
Rhizophilic	High	Low	Glomeraceae[a, b, c, d, e] Claroideoglomeraceae[a] Paraglomeraceae
Edaphophilic	Low	High	Gigasporaceae[a, b, e] Diversisporaceae[a, e]
Ancestral	Low	Low	Archaeosporaceae Ambisporaceae Acaulosporaceae[a, b, e] Pacisporaceae

[a] (Powell et al., 2009).
[b] (Hart and Reader, 2002).
[c] (Varela-Cervero et al., 2015).
[d] (Varela-Cervero et al., 2016a).
[e] (Varela-Cervero et al., 2016b).

In this study, we focus on AMF, but also assess changes in other fungal functional groups including saprotrophs, pathogens and non-AMF symbionts, as these functional groups interact with AMF and are also affected by the same global change drivers (Amend et al., 2015). We hypothesize that: (1) native shrub roots will host relatively more edaphophilic AMF, whereas invasive grass roots will host relatively more rhizophilic AMF; (2) invasive grass roots will harbor fewer pathogens than native shrubs; and (3) elevated soil N concentrations will reduce the richness and relative abundance of edaphophilic AMF taxa. We test these hypotheses within both guild and broader taxonomic frameworks, using high-throughput sequencing coupled with generalized linear models and joint taxa distribution models to understand the importance of multiple environmental variables in structuring fungal communities.

2. Methods

2.1. Site description

We sampled from two chaparral communities in southern California, the San Dimas Experimental Forest (SDEF) and Emerson Oaks Reserve (EOR), both with granitic parent material and coarse sandy loam soils. San Dimas Experimental Forest is in the San Gabriel Mountains (34° 12′ N, 117° 46′ W, 50 km east of Los Angeles), at 830 m above sea level. A small portion of SDEF (~100 ha) was purposely converted from native chaparral to grassland in the 1960s to study the relationship between ecohydrology and community type (Dunn et al., 1988). EOR is in Temecula Valley (33° 28′ N, 117° 2′ W) 500 m in elevation. We sampled in both a grassy patch, ~1 ha, where shrubs had been cleared before the 1980s and in surrounding mature chaparral. Both sites burned in wildfires within the past 20 y (SDEF − 2003, EOR − 2004), and we sampled in both areas where chaparral had recovered, and areas where exotic grasslands persisted. Because of SDEF's proximity to Los Angeles, it receives a large amount of atmospheric N deposition (>19 kg N ha $^{-1}$ yr^{-1}, Fenn et al., 2010). EOR receives much less atmospheric N deposition (~6 kg N ha $^{-1}$ yr^{-1}, Fenn et al., 2010).

2.2. Host plants

In March 2016, we sampled roots and bulk soils at both sites underneath individuals (n = 6) of the dominant native chaparral shrub, *Adenostoma fasciculatum*. *A. fasciculatum* is a dominant shrub species in chaparral which forms several types of root-fungal associations, primarily with AMF, but also with ectomycorrhizal fungi (EMF) and dark-septate fungi (Allen et al., 1999). We sampled the

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dominant invasive grass species (n = 6) at each site (*Bromus diandrus* at EOR and *Avena fatua* at SDEF). Before analyzing samples associated with the invasive grass species as one invasive group, we used a *t*-test to examine differences between them and determined there were no significant differences among richness and relative abundance of OTUs for each locus and could group them. At each site we sampled from adjacent stands (>5 m but <10 m apart) of invasive and native vegetation. Sample size analysis indicated that >95% of fungal richness was likely captured with six samples ('vegan' package, Oksanen et al., 2017).

2.3. Soil sampling

Soil cores were collected at ~10 cm depth from the base of each individual plant. Roots were washed thoroughly with DI water and soils were sieved using a 2 mm mesh that was sterilized with 70% ethanol between samples. Samples were frozen at −20 °C until analyzed. Each soil sample was analyzed for pH in a DI water slurry, for KCl-extractable NH_4 and NO_3 (University of California Davis Analytical Laboratory), and for bicarbonate-extractable P (USDA-ARS Soils Laboratory, Reno, NV). Soil characteristics by site and host plant type are summarized in Table 2.

We extracted DNA from soils (~0.25 g/sample) and roots (~0.15 g/sample) using the PowerLyzer PowerSoil DNA Isolation Kit per manufacturer's protocol (Mo Bio Laboratories, Carlsbad California), with a modified heated lysis step at 65 °C for 20 min, before homogenization (Rubin et al., 2014). Samples were kept frozen at −20 °C and transported on dry ice to the NAU Environmental Genetics and Genomics Laboratory (EnGGen) at Northern Arizona University. Samples were further purified from residual contaminants by the PEG-bead protocol described by Rohland and Reich (2012). DNA concentrations were determined by PicoGreen (Molecular Probes Inc., Eugene OR, USA) fluorescence and standardized to ~10 ng/μL.

2.4. Percent colonization

To assess fungal colonization, roots remaining after DNA extraction were washed from soil, cleared overnight in 2.5% KOH, acidified in 1% HCl, and stained in 0.05% trypan blue (Kormanik and McGraw, 1982; Koske and Gemma, 1989). We estimated percent colonization using a modified magnified intersection method (McGonigle et al., 1990). Roots were mounted in PVLG on microscope slides and 60 intercepts per replicate were observed at 200 × magnification. We examined root fragments for AMF hyphae, arbuscules, vesicles, as well as hyphae, reproductive structures of non-AM fungi, and EMF mantles and Hartig nets.

To test for differences in colonization between invasive and native hosts, five linear models were fitted to percent colonization data using structures listed above as response variables and host plant, site, and host plant by site as the predictor variables. ANOVA was used to assess variable significance. All statistical analyses were performed in R version 3.2.1 (R version 3.2.1; R Core Team, 2017).

2.5. Library construction and sequencing

Samples were amplified by polymerase chain reactions (PCR) for the ribosomal small subunit (SSU) region using the Glomeromycotina-specific AML2 and the universal eukaryote WANDA primer set (Lee et al., 2008; Dumbrell et al., 2011) and for the internal transcribed spacer 2 (ITS2) region using the universal fungal primers 5.8SFun and ITS4Fun (Taylor et al., 2016) in preparation for high-throughput sequencing of the resulting amplicon pools. Library construction was conducted in a two-step procedure as in Berry et al. (2018). First-round amplifications were carried out in triplicate with three separate template dilutions (10 ng, 1 ng, or 0.1 ng template DNA), and with primers possessing universal tails synthesized 5′ to the locus specific sequences (Alvarado et al., 2018). Besides template DNA, reactions contained 0.1 U/μL Phusion HotStart II DNA polymerase (Thermo Fisher Scientific, Waltham, MA), 1X Phusion HF Buffer (Thermo Fisher Scientific), 200 μM dNTPs (Phenix Research, Candler, NC), and 3.0 mM $MgCl_2$. Thermal cycler conditions were as follows: 2 min at 95 °C; 35 cycles of 30 s at 95 °C, 30 s at 55 °C, 4 min at 60 °C; then refrigerate at 10 °C. Triplicate reaction products for each sample were pooled by combining 4 μL from each, and 2 μL was used to check results on a 1% agarose gel. Products were purified by the PEG-bead cleanup and eluted in 100 μL Tris-Cl pH 8.0. 1 μL of purified, diluted product was used as template in a second, indexing PCR reaction, using primers with sequences matching the universal tails at the 3′ end, and matching Illumina MiSeq flowcell sequences at the 5′ end. Conditions for tailing reactions were identical to the first round except that we used 100 nM of each indexing primer, only one reaction was conducted per sample and only 15 total cycles were performed. We used 2 μL to check results on an agarose gel, purified by the PEG-bead cleanup, quantified by PicoGreen fluorescence, and equal masses for every sample were combined into a final sample pool using an automated liquid handling system (PerkinElmer, Waltham, MA). We further concentrated the resulting pool with the PEG-bead protocol, quantified it by qPCR and average fragment sizes were estimated using a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA) prior to sequencing. Sequencing was carried out on a MiSeq Desktop Sequencer (Illumina Inc, San Diego, CA) running in paired end 2 × 300 mode.

Table 2
Soil characteristics for each site (n = 12) and host plant (n = 12). Values shown are mean of all samples with standard error in parentheses.

Source	pH	NH4 (ppm)	NO3 (ppm)	P (ppm)
EOR	6.69 (0.05)	1.51 (0.07)	2.94 (0.60)	11.85 (0.64)
SDEF	6.09 (0.08)	1.76 (0.27)	12.05 (1.89)	7.21 (0.57)
Invasive	6.61 (0.07)	1.31 (0.09)	4.27 (0.96)	9.73 (0.95)
Native	6.19 (0.09)	1.94 (0.24)	10.31 (1.95)	9.54 (0.56)

2.6. Bioinformatics

We used cutadapt (Martin, 2011) to filter sequences for locus-specific primer sequences and SMALT (http://www.sanger.ac.uk/science/tools/smalt-0) to remove residual PhiX contamination, the viral genome used as a control sequence on Illumina Platforms. For the ITS locus, we joined paired-end of raw reads with ea-utils (https://paperpile.com/c/RugXPZ/9eZu, Aronesty, 2011). We then checked joined read quality across read length with FastQC (Andrews, 2010) and trimmed reads with fastq-mcf to remove low quality calls (ITS 291 bp). FastQC: a quality control tool for high throughput sequence data; available online at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc). For the SSU locus, we used the forward raw read and checked quality with FastQC (SSU 201 bp; Andrews, 2010). Demultiplexing was performed in QIIME 1.9.1 (Caporaso et al., 2010) with the split_libraries_fastq.py command using a phred score of 20 (q = 19), allowing zero low-quality base calls (r = 0), and retaining reads only if they possess 95% of initial sequence length following quality truncation (p = 0.95). We screened for chimeras using VSEARCH (Rognes et al., 2016) in uchime_denovo mode for SSU and uchime_ref mode against the UNITE-based fungal chimera dataset for ITS (Nilsson et al., 2015). For ITS2, fungal sequences were extracted using ITSx (Bengtsson-Palme et al., 2013). We picked OTUs using Swarm (Mahe et al., 2014) with a resolution of d4, which collapses sequences with less than 4 differences into a single representative OTU. Taxonomy was assigned using BLAST, with the QIIME default

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e-value of 0.001 (Altschul et al., 1990) against the UNITE ITS reference database (Kõljalg et al., 2013) and MaarjAM database for SSU (Öpik et al., 2010). Reference databases were truncated prior to analysis to include only the region of interest to avoid any spurious results. We further filtered our OTU tables (0.005% across the table) recommended in Bokulich et al., (2013) to remove rare (presumed spurious) OTUs. For both loci, we normalized OTUs using cumulative sum scaling (CSS-normalization) in the metagenomeSeq package of Bioconductor (Paulson et al., 2013) in R prior to further analyses (R Core Team, 2017). CSS normalization attempts to avoid biases in marker gene surveys due to uneven sequencing depth. Read counts are rescaled against a quantile determined by assessing count deviations of each sample as compared to the distribution of counts across all other samples (Paulson et al., 2013). Raw and CSS-normalized OTU tables are available through Mendeley Data at https://doi.org/10.17632/ppmfn3rh7r.1 (Phillips, 2018). Raw sequences have been deposited into the NCBI Short Read Archive (SRA) and can be accessed here: https://www.ncbi.nlm.nih.gov/sra/PRJNA507491.

2.7. Functional group assignment

To examine responses of the general fungal community (ITS2), we assigned OTUs to functional groups using the online application FUNGuild ("http://www.stbates.org/guilds/app.php", Nguyen et al., 2016). After processing OTUs through FUNGuild, we removed Glomeromycotina from the symbiont group to remove redundancy of ITS2 and SSU sequences. The remaining non-AMF symbionts includes EMF. EMF occurrence was low in both native and invasive samples; therefore we did not analyze them separately. To simplify, FUNGuild functional groups 'pathotrophs', 'pathotroph-saprotrophs' and 'pathotroph-symbiotrophs' were assigned to the pathogen group; and 'saprotrophs' and 'saprotroph-pathotroph' to the saprotroph group. We kept only FUNGuild assignments that were at the confidence level of 'highly probable' and 'probable', removing all taxa that were at the confidence level of 'possible' for these analyses. We retain saprotrophic FUNGuild assignments in roots under the assumption that these saprotrophs may be opportunistically parasitizing plant roots, as recent research uncovers the potential for fungi to occupy multiple niches (Glynou et al., 2017; Selosse et al., 2018). With these constraints, FUNGuild was able to assign function to 585 OTUs (62%) of 940 ITS2 OTUs.

For the SSU locus, 181 OTUs (65%) out of 277 were assigned taxonomy by using BLAST against the MaarjAM database. We manually BLASTed the 'no blast hits' against the NCBI database to ensure that these OTUs were not Glomeromycotina. Therefore, we did not retain the 96 OTUs (35%) with 'no blast hit' in any of our downstream analyses. To interpret responses of the AMF community (SSU) we assigned families of Glomeromycotina to AMF functional groups: rhizophilic, edaphophilic and ancestral using AMF resource allocation patterns defined in previous studies (Table 1). Families that did not fall into rhizophilic or edaphophilic groups were placed in the ancestral group (Table 1). We did not include sequences reportedly identified as *Geosiphon pyriformis*, of which there were only two observations, in any of the functional groups.

2.8. Beta diversity

For each locus, we visualized beta-diversity using non-metric multidimensional scaling (NMDS) of the Bray-Curtis distances, using distance matrices generated from CSS-normalized data before filtering for functional group assignment. The NMDS was visualized in R (R version 3.2.1; R Core Team, 2017) using the ggplot2 package (Wickham 2009) and the 'stat_ellipse' function with 95% confidence intervals. We tested for differences in overall general fungal (ITS2) and AMF (SSU) community composition across treatments by performing permutational multivariate ANOVA (PERMANOVA) for each locus using the 'adonis' function in the 'vegan' R package (999 permutations; Oksanen et al., 2017). Host plant, site, type (root or soil), pH, NO_3, NH_4, and P were used as the predictor variables. For the SSU locus, we could not include pH, NO_3, NH_4 and P in the PERMANOVA because the multivariate homogeneity of groups dispersion was not met. For the ITS2 locus, we could include all variables as the homogeneity of groups dispersion was met for every predictor variable.

2.9. Generalized linear models

We used generalized linear models (GLMs) to test our hypotheses about fungal functional group responses to invasion and elevated soil N concentrations. We built GLMs using the 'glm' function in the MASS package in R (Venables and Ripley, 2002). We fitted models using gaussian, negative binomial, poisson and log normal distributions where appropriate, determined with the 'qqp' function in the MASS package to visually assess probability distribution fit. We used the 'stepAIC' function from the MASS package to further select these models for parsimony (Venables and Ripley, 2002). We used separate models for roots and soils by functional group richness and relative abundance of each locus, resulting in twenty-four models.

2.10. Joint taxa distribution modeling

To understand how environmental variables structure AMF relative taxonomic abundance, we analyzed read abundance data (Paulson et al., 2013) using joint distribution models following the Hierarchical Modeling of Species Communities approach ('HMSC' R package) as outlined in Ovaskainen et al. (2017). The HMSC approach uses a hierarchical Bayesian structure to fit a joint distribution model to presence/absence or abundance data of taxa from diverse communities.

We built and evaluated models examining responses of AMF read abundance for roots and soils of the SSU locus at the family level, resulting in two models. We performed 200,000 Markov chain Monte Carlo (MCMC) iterations of each model, of which the first half was discarded, and the remaining 100,000 were further thinned, resulting in 1000 posterior samples. We used flat priors and sampled the posterior distribution using the Gibbs sampler with a Gaussian distribution. Both models included the same environmental predictors: host plant, site, pH, NH_4, NO_3, and P. We considered environmental predictors as fixed effects and individual sample as a random effect. We checked for model convergence by visually assessing the MCMC trace plots. We used the posterior distributions of each predictor and calculated the probability that it was different from zero. We considered parameters "significant" when their posterior probabilities had at least a 90% probability of being different from zero ($p = 0.1$). We used the 'variPart' function in the HMSC package to calculate the relative proportion of the total model variance that is attributable to each of the fixed and random effects (Blanchet and Tikhonov, 2016). This allows us to assess the explanatory power of our models, while also understanding how much variation in family abundance can be explained by each of our environmental variables as well as random processes.

3. Results

3.1. Percent colonization

Roots of invasive annual grasses had higher colonization by AM and non-AM hyphae than native shrub roots (72% ± 4 (mean ± SD)

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invasive and 5% ± 33 native, P = 0.003, and 56% ± 38 and 8% ± 7, P = 0.023, respectively). Rates of AMF hyphal colonization in roots were higher in both native and invasive host plants at SDEF than at EOR (55% ± 35 vs. 13% ± 11). The colonization of arbuscules (0% in native and 1% in invasive roots) was too low to analyze statistically, though we did observe more vesicles in invasive roots than in native roots (11% and 2%, respectively; P = 0.002). We did not observe EMF colonization in *A. fasciculatum* roots.

3.2. SSU sequences (AMF)

We observed a total of 277 OTUs, 181 of which were assigned taxonomy after performing BLAST against the MaarjAM database. For sequences with assigned taxonomies, we observed a mean of 335 ± 121 (SD) reads, and 52 ± 16 OTUs, per sample. These OTUs belonged to 3 orders, 10 families and 9 genera within Glomeromycotina. We observed the following 9 genera: *Glomus, Acaulospora, Archaeospora, Paraglomus, Scutellospora, Claroideoglomus, Geosiphon, Ambispora,* and *Redeckera*. Of those genera, only 2 OTUs were identified as *G. pyriformis* which we removed from subsequent analyses, because it did not fall into any AMF functional grouping. Family relative read abundances can be found in Table S2. We placed these OTUs into three functional guilds described earlier (Table 1). Of these guilds, the most common were rhizophilic AMF (264 ± 105 reads and 39 ± 12 OTUs per sample), followed by edaphophilic families (50 ± 29 reads and 8 ± 3 OTUs per sample) with ancestral AMF being the least common (39 ± 20 reads and 16 ± 6 OTUs per sample).

3.3. ITS2 sequences (general fungal community)

We observed a mean ± SD of 661 ± 277 reads and 125 ± 50 OTUs per sample. These OTUs belonged to 7 phyla, 21 classes, 40 orders, 79 families and 149 genera. The most abundant phylum in the roots was Ascomycota with 442 ± 203 reads and 84 ± 32 OTUs per sample, followed by Basidiomycota with 182 ± 104 reads and 33 ± 18 OTUs. Saprotrophs were the most common (189 ± 219 reads and 36 ± 42 OTUs per sample), followed by pathogens (65 ± 64 reads and 13 ± 11 OTUs per sample) and non-AMF symbionts (62 ± 65 reads and 11 ± 8 OTUs per sample). Once we had removed AMF to avoid overlap between our datasets, the remaining fungal symbionts consisted of 11 families, 11 genera, and 20 species. Of the 11 families, seven families – Inocybaceae, Tricholomataceae, Pyronemataceae, Sclerodermataceae, Helvellaceae, Rhizopogonaceae and Paxillaceae – contain EMF species. Four families – Collemataceae, Teloschistaceae, Lobariaceae, Lecideaceae – contain lichenized fungal species.

3.4. Beta diversity

AMF beta diversity differed by site ($R^2 = 0.04$, P = 0.02, Fig. 1). Host plant, sample type (root or soil) and their interaction did not significantly structure AMF beta diversity ($R^2 = 0.01$ and 0.02; P = 0.9 and 0.6, respectively). Beta diversity of the general fungal community was significantly structured by host plant ($R^2 = 0.04$, P = 0.01, Fig. 2) and the interaction between host plant and sample type ($R^2 = 0.03$, P = 0.04, Fig. 2).

3.5. Functional group responses

3.5.1. Rhizophilic AMF

Richness and relative read abundance of rhizophilic AMF was greater in native than invasive roots (P = 0.008 and 0.02, $R^2 = 0.81$ and 0.82, respectively; Fig. 3A; Table S1). Rhizophilic AMF richness and relative abundance in roots was negatively correlated with soil

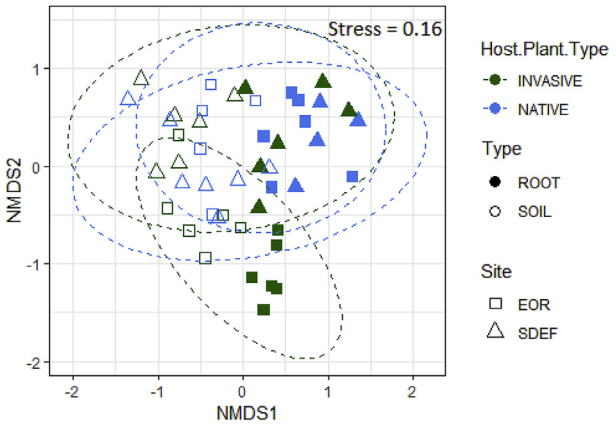

Fig. 1. AMF (SSU) Bray-Curtis NMDS plot. Color is host plant, shape denotes site: San Dimas Experimental Forest (SDEF) or Emerson Oaks Reserve (EOR) and fill denotes if the community is from a root (solid) or soil (no fill) sample. The stress value is 0.16.

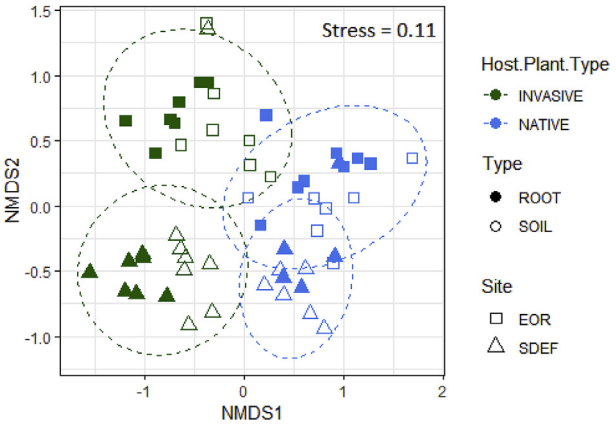

Fig. 2. General Fungal Community (ITS2) Bray-Curtis NMDS plot. Color is host plant, shape denotes site: San Dimas Experimental Forest (SDEF) or Emerson Oaks Reserve (EOR) and fill denotes if the community is from a root (solid) or soil (no fill) sample. The stress value is 0.11.

NH_4 concentrations (P = 0.003 and 0.016, $R^2 = 0.81$ and 0.82, respectively; Table S1). Rhizophilic AMF richness and relative read abundance in roots were positively associated with soil NO_3 concentrations (P = 0.01 and 0.002, $R^2 = 0.81$ and 0.82, respectively; Table S1). There were no differences in the richness or relative abundance of rhizophilic taxa in soils underneath native shrubs and invasive grasses (P = 0.71 and 0.77, $R^2 = 0.21$ and 0.15, respectively; Fig. 3B).

3.5.2. Edaphophilic AMF

The relative abundance of edaphophilic AMF was higher in native shrub roots than in invasive grass roots (P = 0.02, $R^2 = 0.69$, Table S1), while richness did not differ between these plant roots (P = 0.26, $R^2 = 0.60$, Fig. 3A). The richness of edaphophilic AMF in soils underneath native shrubs and invasive grasses did not differ (P = 0.77, $R^2 = 0.12$), however edaphophilic AMF were relatively more abundant in native soils (P = 0.007, $R^2 = 0.65$, Table S1). Richness of edaphophilic AMF in roots was positively correlated with soil NO_3 (P = 0.04, $R^2 = 0.60$, Table S1). Relative abundance of edaphophilic AMF in soils was negatively correlated with soil NH_4 concentrations and positively correlated with soil NO_3 concentrations (P = 0.03 and 0.005, $R^2 = 0.65$ and 0.12, respectively; Table S1).

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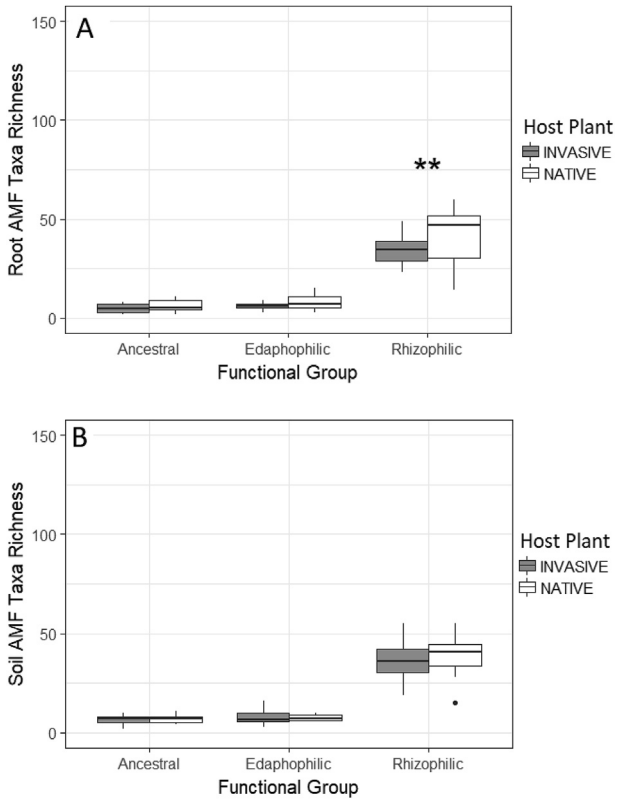

Fig. 3. SSU or arbuscular mycorrhizal fungal (AMF) root (A) and soil (B) communities by functional group by aggregating species by family using the phylogenetic scheme in Table 1. AMF taxa richness is the number of times a unique taxonomic unit is encountered in each sample. *** denotes significant difference by host plant type at $P < 0.001$, ** denotes significance at $P < 0.01$ and * denotes significance at $P < 0.05$ from GLM outputs in Table S1.

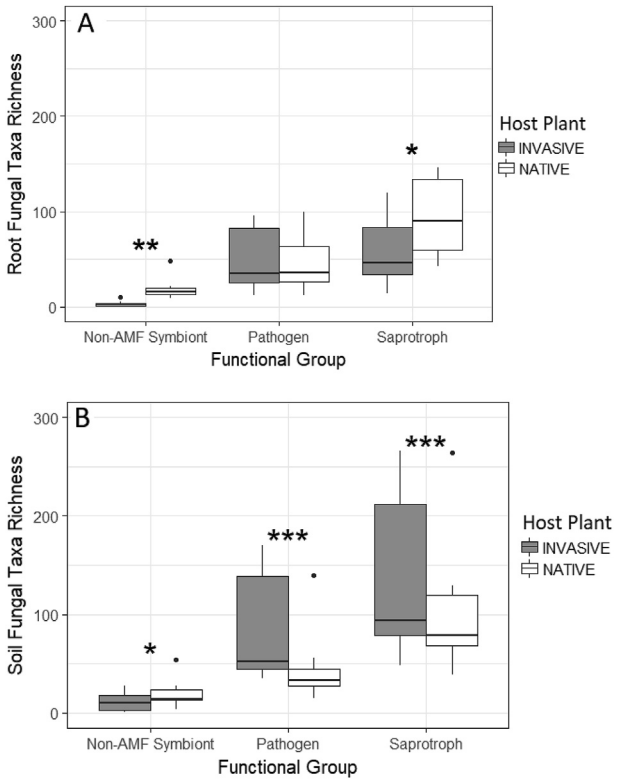

Fig. 4. ITS or general fungal community root (A) and soil (B) communities by functional group by aggregating species using FUNGuild. Fungal taxa richness is the number of times a unique taxonomic unit is encountered in each sample. *** denotes significant difference by host plant at $P < 0.001$, ** denotes significance at $P < 0.01$ and * denotes significance at $P < 0.05$ from GLM outputs in Table S1.

3.5.3. Ancestral AMF

Native roots had greater relative read abundance, but not richness of ancestral AMF families when compared to invasive grass roots ($P = 0.006$ and 0.2, $R^2 = 0.76$ and 0.66, respectively; Table S1). Host plant was not included in the ancestral soil relative abundance and richness models after model selection. Root ancestral AMF richness was negatively correlated with soil NH_4 concentrations and positively associated with soil NO_3 concentrations ($P = 0.01$ and 0.01, $R^2 = 0.66$, Table S1). Conversely, soil ancestral AMF richness and relative read abundance were negatively associated with increased soil NO_3 concentrations ($P = 0.003$ and 0.03, $R^2 = 0.44$ and 0.40, respectively; Table S1).

3.5.4. Non-AMF symbionts

Non-AMF symbionts – including EMF – had greater richness (Fig. 4A) and relative abundance in native roots ($P = 0.002$ and 0.003, $R^2 = 0.95$ and 0.98, respectively; Table S1). Non-AMF symbiont richness, but not abundance, was also greater in native soils (Fig. 4B, $P = 0.035$ and 0.13, $R^2 = 0.95$ and 0.98, respectively; Table S1). Non-AMF symbiont richness in roots was negatively associated with soil NH_4 and NO_3 concentrations ($P = 0.001$ and 0.001, respectively, $R^2 = 0.95$; Table S1). Conversely, non-AMF symbiont relative abundance was positively associated with soil NH_4 and NO_3 soil concentration ($P = 0.001$ and 0.003, respectively, $R^2 = 0.98$; Table S1).

3.5.5. Pathogens

Pathogen fungi were relatively more abundant in invasive grass roots ($P = 0.011$, $R^2 = 0.58$; Table S1), however richness did not differ (Fig. 4A, $P = 0.63$, $R^2 = 0.60$). Pathogen richness (Fig. 4B) and relative abundance were greater in invasive soils ($P = 0.001$ and 0.001, $R^2 = 0.84$ and 0.82, respectively; Table S1). SDEF had higher pathogen richness and relative abundance in soils than EOR ($P = 0.001$ and 0.001, $R^2 = 0.84$ and 0.82, respectively; Table S1). SDEF had higher pathogen richness and relative abundance in soils than EOR ($P = 0.001$ and 0.001, $R^2 = 0.84$ and 0.82, respectively; Table S1).

3.5.6. Saprotrophs

Saprotroph relative abundance was greater in invasive soils ($P = 0.001$, $R^2 = 0.73$), and saprotroph richness was greater in invasive soils ($P = 0.001$, $R^2 = 0.65$, Fig. 4B; Table S1). Richness and relative abundance of saprotrophs in soils were positively associated with higher soil NH_4 concentration ($P = 0.001$ and 0.001, $R^2 = 0.65$ and 0.73, respectively; Table S1). Saprotroph richness in soils negatively correlated with soil NO_3 concentration ($P = 0.022$, $R^2 = 0.65$; Table S1). Root saprotroph richness was higher in native roots when compared to invasive ($P = 0.03$, $R^2 = 0.54$; Fig. 4A, Table S1).

3.6. Taxonomic abundance responses

3.6.1. AMF families

The relative abundance of AMF families did not vary significantly between the roots nor soils beneath invasive grasses and native shrubs (Tables S4 and S5). Taxa belonging to Archaeosporaceae, Claroideoglomeraceae, Diversisporaceae, and Glomeraceae were relatively more abundant in roots at EOR ($P \leq 0.1$,

Table S4), however we found no significant differences between sites in soils (Table S5). Relative read abundance for all AMF families in roots was positively correlated with soil NO_3 concentrations ($P \leq 0.1$, Table S4). We observed increases in relative abundance of Acaulosporaceae, Archaeosporaceae, Claroideoglomeraceae, Diversisporaceae, Glomeraceae, and Paraglomeraceae in roots with increasing soil P concentrations ($P \leq 0.1$, Table S4). In soils, fewer environmental variables were significantly associated with relative abundance of AMF families. Relative abundance of taxa belonging to: Acaulosporaceae, Archaeosporaceae, Diversisporaceae, and Paraglomeraceae were positively associated with soil pH concentrations ranging from 6 to 7 ($P \leq 0.1$, Table S5). Relative abundance of Acaulosporaceae, Ambisporaceae, and Claroideoglomeraceae in soils increased with increasing soil NH_4 concentrations ($P \leq 0.1$, Table S5).

3.6.2. Variance partitioning

Environmental predictors (host plant, site, NH_4, NO_3, pH, and P) explained 92% ± 7% of the variance in the AMF root community model (Fig. 5A, Table S6). Relative abundance of Ambisporaceae in roots, which was more abundant in native samples, had the most model variance explained by host plant, 19%, and for all other AMF families host plant explained less than 10% of model variance (Table S6, Fig. 5A). Soil NO_3 concentrations explained the largest amount of model variance in the root model (33% ± 4%, Fig. 5A, Table S6). In soil communities, total environmental predictors explained 92% ± 7% of model variance (Fig. 5B, Table S7). Soil P concentrations explained the largest amount of the variance ranging from 35% ± 14% of the variation in the soil model (Fig. 5B, Table S7).

4. Discussion

Overall our findings suggest that while the same pool of AMF mutualists is available for both *A. fasciculatum* and the invasive grasses we sampled, the mycorrhizal communities of these plants differ, potentially because of differences in plant roots and fungal biomass allocation (Maherali and Klironomos, 2007; Powell et al., 2009; Sikes et al., 2009, 2010). The increased proportion of edaphophilic AMF among native shrub roots and soils provides some support for our first hypothesis, and is consistent with other studies in which locally adapted fungi exhibit a preference for locally adapted host plants (Johnson et al., 2009). However, this finding is contrary to our microscopic observations of higher AMF colonization in invasive roots than native roots. We expected that invasive grasses would host more rhizophilic AMF taxa, however these taxa were relatively more abundant and richer in native shrub roots. We hypothesized that invasive grasses would harbor fewer pathogens but did not find strong support for this. Instead, we found that pathogenic fungi were relatively more abundant in invasive roots

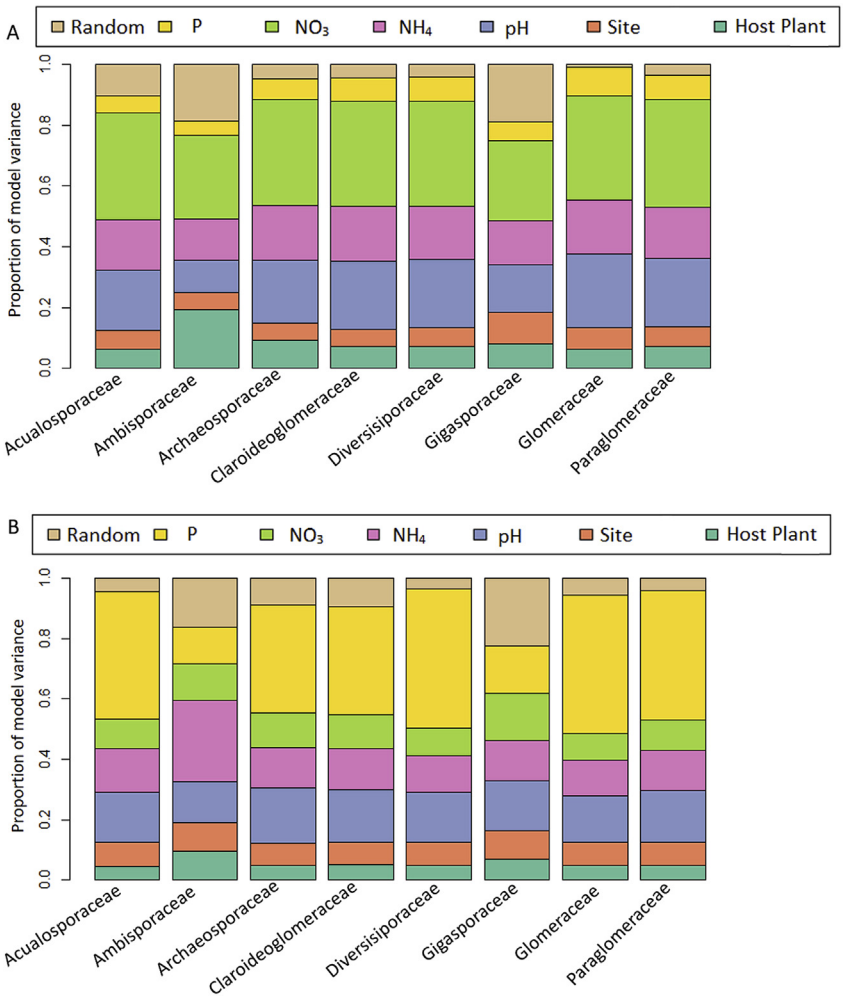

Fig. 5. Results of variance partitioning for the variation in root (A) and soil (B) AMF relative abundance (at the family level) in response to host plant (native or invasive), site (SDEF or EOR), P, NO_3, NH_4, pH, site, and host plant. Individual sampled was included as the random effect.

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and soils. Microscopic observations showed that invasive grass roots were colonized by both AMF and non-AMF at higher rates than the roots of the native shrub *A. fasciculatum*. We expected that invasive hosts would interact with soil N, resulting in decreased richness and abundance of edaphophilic AMF, but we have little support for this hypothesis. While our beta-diversity analyses suggest that habitat filtering alters AMF abundances between soils and roots, we observed an even greater separation between the rest of the fungal community between native and invasive plant roots.

4.1. Symbiotic fungi

Lower richness and relative abundance of some AMF functional groups in invasive roots, concurs with past research suggesting that invasive annual grasses may be less dependent on AMF mutualisms (Allen, 1984; Richardson et al., 2000; Callaway et al., 2004; Reinhart and Callaway, 2006; Busby et al., 2011, 2013). If invasive grasses are less dependent on soil mutualists, this could facilitate rapid establishment of these grasses following disturbance. The degraded mutualist hypothesis suggests that invasive plant species that successfully establish due to decreased dependence on soil mutualisms will decrease the presence of plant species that are highly dependent on mutualisms over time (Vogelsang and Bever, 2009). We found relative decreases in three groups of soil symbionts associated with invasive host plants: non-AMF symbionts (including EMF), edaphophilic and rhizophilic AMF. This suggests that persistence of the invasive grasses we sampled may decrease the presence of multiple soil symbionts that native species depend on for pathogen protection and for increased access to soil resources.

In invasive roots, we observed lower relative abundance coupled with lower richness for some groups of AMF compared to native roots, which may result in losses of necessary function and/or taxa native plants rely on. Specifically, decreases in proportions of edaphophilic AMF would decrease the presence of extraradical hyphae that *A. fasciculatum* depends on for resource uptake. These results, combined with no change in richness associated with invasion, align with previous findings in the literature that variation in AMF composition between systems is often due to differences in abundance rather than a distinct taxonomic composition (Hijri et al., 2006; Öpik et al., 2008; Hart et al., 2016). This suggests that when these invasive grasses persist, we may see shifts in the relative abundance of taxa, but not a complete turnover of AMF taxa that are present. However we also observed greater AMF colonization in invasive than native roots which may confer greater pathogen protection (Maherali and Klironomos, 2007; Sikes et al., 2009). Microscopic observations of *A. fasciculatum* included a range of root diameters, while we only used the finest root tips for sequencing, which likely have higher colonization (Allen, 2001). Another study reported higher rates of AMF colonization in *A. fasciculatum* as well as EMF in wet but not dry years (Allen et al., 1999). We sampled during a drought year which likely decreased the presence of AMF and EMF in these soils.

We did not observe effects of site or host plant on any AMF families in roots or soils, but in our functional guild analyses we found that rhizophilic and edaphophilic AMF were relatively more abundant in native roots. This indicates that the complexity of family-level community composition may be effectively reduced using a functional grouping approach, allowing nuanced relationships between invasion and AMF communities to be resolved at this scale. However, variance partitioning from family-level analysis indicated that environmental variables differentially structure AMF root and soil communities which agrees with our beta diversity results. For soils, the largest amount of variability across all AMF families was attributed to soil P concentrations. However, less variability was explained for Gigasporaceae and Ambisporaceae abundance by soil P compared to other AMF families. The Gigasporaceae family falls into the edaphophilic AMF group, but the Diversisporaceae, the other family in this group, has much more variability explained by soil P. This may mean that responses to environmental variables are not consistent across resource allocation strategies of AMF, or that we still need a better understanding of resource allocation of some families.

For roots, the largest amount of variability across all AMF families was attributed to soil NO_3 concentrations, meaning that selectivity of the host plant and fungi in initializing mutualisms may heavily depend on this. We observed relative increases in abundance for most AMF families with increased soil NO_3. Specifically, Glomeraceae and Paraglomeraceae (rhizophilic) appear to be the most positively associated with the higher soil NO_3 concentrations, whereas Gigasporaceae (edaphophilic) and Ambisporaceae (ancestral) showed little increase with elevated NO_3, a pattern that was also observed by Egerton-Warburton and Allen (2000) and Treseder et al. (2018). This agrees with previous research demonstrating that AMF which produce extensive extraradical hyphae respond negatively to soil N concentrations, while those which colonize roots intensively are stimulated by increasing soil N concentrations (Egerton-Warburton et al., 2007). We must note that the family level results from our joint distribution model need to be interpreted cautiously, because we use relative read abundances in these models. The read abundance data we use is CSS-normalized, which accounts for multiple common issues including under sampling and amplification bias (Paulson et al., 2013), however it is important to acknowledge estimating biological abundance from sequence read numbers remains imperfect (Weiss et al., 2017). While imperfect, read abundance data still has the potential to provide information about how environmental conditions structure microbial communities (Ghanbari et al., 2017; Timonen et al., 2017; Collins et al., 2018).

Our results suggest that differences in richness and relative abundance of symbionts, both AMF and non-AMF, may be associated with host plant identity. Non-AMF symbionts detected by ITS2 sequencing were mainly EMF indicating their presence even though they were not detected microscopically. Nevertheless, *A. fasciculatum* forms EMF under wet conditions (Allen et al., 1999), and invasive grass encroachment may indirectly decrease EMF colonization by rapidly depleting soil moisture (Melgoza et al., 1990). It may be important to understand the richness and abundance of different functional groups of fungi in natural recolonization or restoration efforts of slow-growing shrubs like *A. fasciculatum*, that could be highly dependent on locally diverse adapted symbiotic relations for establishment (Azcón-Aguilar et al., 2003; Johnson et al., 2009).

4.2. Pathogenic and other non-AMF fungi

We did not find evidence to support the hypothesis of pathogen release in this system (Mitchell and Power, 2003; Kardol et al., 2007; Van Grunsven et al., 2007; Reinhart et al., 2010), as pathogen relative abundance was greater in invasive roots and soils. SDEF had a greater richness of pathogens than EOR, which may be related to increased soil N availability at SDEF. Additionally, we observed greater relative abundance of rhizophilic AMF in soils and richness in roots at SDEF which may promote greater pathogen protection (Maherali and Klironomos, 2007; Sikes et al., 2009). It is important to note that in using FUNGuild to assign functional groups while also filtering out all taxa with the confidence level 'possible' (Nguyen et al., 2016), we lost potentially valuable data. However, using only conservative functional group assignments with the confidence levels 'highly probable' and 'probable'

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protected the integrity of our interpretations. There was an increase in non-AMF colonization in invasive roots that could be due to increased pathogen or saprotrophic colonization. This was also supported by ITS2 data, which showed significant differences in pathogen and saprotrophic richness or relative abundance in invasive grass roots.

Recent research suggests that some fungi may have the potential to occupy complex or multiple niches (Glynou et al., 2017; Selosse et al., 2018). Our findings of greater potential saprotroph richness in living *A. fasciculatum* roots support this by indicating that some fungi could be acting as opportunistic pathogens or endophytes. The idea that fungi possess dual niches stems from the evolutionary propensity of fungi to shift ecological niches, while often retaining their previous niche (Selosse et al., 2018). Therefore, these presumably saprotrophic fungi may be acting as facultative pathogens in roots and saprotrophs in soils. Additionally, invasive annual grasses produce larger amounts of easily decomposed litter, which helps to explain our observations of greater relative abundance of saprotrophs in invasive associated soils (De Deyn et al., 2008).

We used FUNGuild and a recently developed AMF guild framework to assign function to fungal taxa, to aid understanding of the ecological relevance of taxonomic differences between host plants and across environmental conditions. Out of necessity for interpretation, both methods constrain descriptions of fungal function to simple categories. Despite this need, it is important to remember that interactions between fungi and plant hosts are complex, varying within taxa and individuals, with the potential to occupy multiple ecological niches under varying environmental conditions (Selosse et al., 2018). Thus, both the AMF guild framework and the FUNGuild application that we use in this study are coarse tools which at best approximate fungal ecological functioning. Our approach is supported by Treseder et al. (2018), who found that high soil N was negatively related to external hyphal length. The use of sequencing data to understand fungal ecology is ultimately limited by research that links fungal life histories and ecological functioning to sequence data.

5. Conclusions

Invasive grasses had lower richness and abundance of both AMF and non-AMF symbionts compared to native shrubs, suggesting that type conversion from native shrubland to non-native grasses may decrease the richness and abundance of some symbiotic fungal taxa in soils (Hawkes et al., 2006; Busby et al., 2011, 2013). Yet, this must be interpreted cautiously because our AMF colonization contradicts this finding because it suggests that AMF are more abundant in invasive roots. We observed differences in relative abundance and richness of functional groups of AMF between native and invasive root and soil communities. However, in our taxonomic analyses we did not find differences in abundance of any AMF family between native and invasive roots or soils. Our results show some support for the hypothesis that native shrubs host a more abundant (but not richer) community of edaphophilic AMF. Decreases in available edaphophilic AMF taxa may hamper the re-establishment of native shrubs into their home range by decreasing access to host-specific mutualists (Johnson et al., 2009). Our results do not support our hypothesis that invasive grasses would host more rhizophilic taxa, as rhizophilic AMF were richer and relatively more abundant in native shrub roots. However, we did observe a larger amount of both AMF and non-AMF colonization in invasive grass roots.

Previous work on soil fungal communities and invasion provides evidence in support of pathogen release in other systems (Mitchell and Power, 2003; Kardol et al., 2007; Van Grunsven et al., 2007; Reinhart et al., 2010). Our hypothesis that pathogen release is promoting high abundances of invasive plants in chaparral is contradicted by higher relative abundances of pathogens in invasive plant roots, coupled with higher rates of non-mycorrhizal root colonization. The higher relative abundances of these potentially parasitic fungi in invasive grass roots compared to native shrubs may be a result of density dependence, given that invasive grasses occur at higher densities than native shrubs. Future work should: (i) aim to confirm that these potential parasites negatively affect invasive plants; and (ii) investigate invasive plant and parasitic fungal abundance dynamics over multiple seasons.

We did not find strong support for our hypothesis that elevated soil N concentrations would reduce the relative abundance of edaphophilic AMF. Surprisingly, edaphophilic AMF richness was positively correlated with soil NO_3 concentrations. However, we did observe decreased relative abundance of edaphophilic AMF associated with invasive hosts relative to native hosts. Future work should include experimental manipulation of soil N and invasion to better resolve the relationship between N availability, exotic plant invasion, and AMF composition. Our results illustrate the importance of including both microscopic observations and sequencing data in efforts to understand AMF. There is a need for more information about the relationship between taxonomy and function of both AMF and other fungi, to address how the interplay of fungi and plants will shift in response to global change.

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Supplementary data

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