Applied Soil Ecology xxx (xxxx) xxxx

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Native and invasive inoculation sources modify fungal community assembly and biomass production of a chaparral shrub

Michala L. Phillips[a,*], Emma L. Aronson[b], Mia R. Maltz[b], Edith B. Allen[a]

[a] Department of Botany and Plant Sciences, University of California Riverside, 900 University Ave., Riverside, CA 92521, United States
[b] Department of Microbiology and Plant Pathology, University of California Riverside, 900 University Ave., Riverside, CA 92521, United States

ARTICLE INFO

Keywords:
Restoration
California chaparral
AMF
Plant invasion
Mycorrhizae
Rhizophilic AMF

ABSTRACT

Feedbacks between plants and surrounding soil microbes can contribute to the establishment and persistence of invasive annual grasses as well as limit the success of restoration efforts. In this study, we aim to understand how three sources of soil inocula – native, invasive (from under *Bromus diandrus)* and sterile – affect the growth response and fungal community composition in the roots of a chaparral shrub, *Adenostoma fasciculatum*. We grew *A. fasciculatum* from seed in a greenhouse with each inoculum source and harvested at six months. We measured above- and below-ground biomass, arbuscular mycorrhizal fungal (AMF) colonization and conducted targeted-amplicon sequencing of the 18S and ITS2 loci to characterize AMF and general fungal community composition, respectively. Native inoculum resulted in roots with richer communities of some groups of AMF and non-AMF symbionts, when compared to roots grown with invasive or sterile inoculum. Seedlings grown with invasive and native inoculum did not have different growth responses, but both produced more biomass than a sterile control. These findings suggest that inoculation with soil from native species can increase the diversity of multiple groups of fungal symbionts and inoculation with live soil (invasive or native) can increase seedling biomass. Moreover, future work would benefit from assessing if a more diverse community of fungal symbionts increases seedling survival when planted in field restoration sites.

1. Introduction

Terrestrial plant invasion by exotic annual grasses has been a persistent ecological challenge facing land managers for quite some time (D'Antonio and Vitousek, 1992). California's predominant vegetation type, chaparral, was thought to be resilient to disturbance and resistant to invasion (Minnich and Bahr, 1995; Allen et al., 2018), yet has recently undergone invasion in part due to increases in fire frequency (Meng et al., 2014; Dickens and Allen, 2014; Stylinski and Allen, 1999; Keeley and Brennan, 2012). Vegetation-type conversion occurs when one vegetation type replaces another, such as conversion from evergreen shrubland to exotic annual grasses has cascading effects on ecosystem function and services provided by chaparral plant communities (Williamson et al., 2004). As type conversion increases in the chaparral, practical strategies for active restoration of these communities are needed (Allen et al., 2018).

Future restoration efforts in the chaparral will likely rely on nursery-grown transplants as seeding efforts have had poor success (Stratton, 2005; Allen et al., 2018); therefore, it is important to examine the growth response of native seedlings grown with different sources of inocula in the nursery. More specifically, feedbacks between plants and soil biota are known to play key roles in structuring plant communities (Wardle et al., 2004; van der Putten et al., 2013). Invasive grasses may be able to persist due to *a priori* presence of mutualistic and freedom from host-specific pathogenic soil fungi or by altering the belowground community (Reinhart and Callaway, 2006; Pringle et al., 2009; van der Heijden et al., 2008; Hilbig and Allen, 2015). When an invasive plant enters a native community, it alters aboveground inputs to the soil (e.g. decomposable litter or the amount of photosynthates directed towards mycorrhizal fungi) which in turn can alter belowground community composition and function (Wolfe and Klironomos, 2005; Reinhart and Callaway, 2006; Inderjit and van der Putten, 2010). In environments with limited water and/or nutrient availability, plants are often heavily dependent on mycorrhizal relationships, meaning that disruptions of mutualistic networks through invasion could promote the establishment and persistence of invasive plants (Richardson et al., 2000; Callaway et al., 2008; Busby et al., 2013). Furthermore, if invasive annual grasses are altering and conditioning soils then these soils may not be an appropriate choice for propagating chaparral plants for restoration.

* Corresponding author.
E-mail address: michala.phillips@gmail.com (M.L. Phillips).

https://doi.org/10.1016/j.apsoil.2019.103370
Received 20 April 2019; Received in revised form 27 September 2019; Accepted 30 September 2019

In addition to potential disruptions of mutualistic networks by invasive grasses, there are inherent differences in life history traits between native perennial shrubs and the annual grasses that are replacing them. Invasive annual grasses possess fibrous short-lived roots which may mean they are less dependent on mycorrhizal fungi (Busby et al., 2011; Owen et al., 2013), whereas chaparral shrubs possess longer-lived coarser roots that are more dependent on mycorrhizal symbioses for water and nutrient uptake (Chen and Brassard, 2013). In two studies, invasive grass neighbors (*Bromus hordeaceus* and *Avena barbata*) altered the community composition of arbuscular mycorrhizal fungi (AMF) found colonizing native roots, resulting in AMF community composition more similar to invasive-associated communities than to natives grown alone (Nelson and Allen, 1993; Hawkes et al., 2006).

This annual life cycle of *Bromus* species may lead to associations with an AMF community composed of families that are relatively rapid colonizers and that produce mainly intraradical hyphae, such as in Glomeraceae (Allen et al., 2003; Maherali and Klironomos, 2007). Whereas native perennials will likely be more dependent on AMF families that colonize more slowly but produce a larger amount of extraradical foraging hyphae that are associated with increased nutrient acquisition such as in Gigasporaceae (Hart and Reader, 2002; Allen et al., 2003; Maherali and Klironomos, 2007). This dichotomy of biomass allocation strategies between families of AMF was used to develop a guild approach to classify AMF families (Weber et al., 2019; Phillips et al., 2019) as 'edaphophilic,' with high allocation to extraradical hyphae; 'rhizophilic,' with high allocation to root colonization; or as 'ancestral' with lower allocation to either root colonization or soil hyphae than the edaphophilic or rhizophilic guilds (Table S1). Families in the edaphophilic guild produce extraradical hyphae to increase the host plant access to nutrients and water, whereas rhizophilic families have the potential to confer pathogen protection to their hosts via intraradical colonization (Weber et al., 2019; Phillips et al., 2019). Using this guild approach, we can assess if and how invasive grasses disrupt mycorrhizal communities and uncover the consequences for native plants cultivated in either invasive- or native plant-conditioned soils.

In this study, we propagate a chaparral shrub, *Adenostoma fasciculatum*, from seed in a greenhouse with inoculum collected from native and invasive conditioned soils, as well as a sterile control. We chose *A. fasciculatum* for three reasons: it is one of the most commonly occurring species in the chaparral, it is the dominant species surrounding the invaded portion of our field site, and it has the potential not only to increase AMF presence in the soil, but also ectomycorrhizal fungal (EMF) diversity and abundance because it may form both types of mycorrhizae (Allen et al., 1999). We hypothesize that (1) native seedlings grown with invasive inoculum will have lower rates of AMF and non-AMF colonization compared to those grown with native inoculum; (2) the fungal communities colonizing the roots of the native seedings grown with invasive inoculum will be less diverse than those grown in native conditioned soils; (3) seedlings grown with invasive and sterilized inoculum will produce relatively less biomass than seedlings grown with native inoculum. To test these hypotheses, we combined a greenhouse experiment with high-throughput sequencing of soil fungal communities to determine if plant-soil feedbacks from invasive conditioned soils would hamper the growth of chaparral seedlings.

2. Methods

2.1. Greenhouse experiment

Soils were collected from Emerson Oaks Reserve located in Temecula Valley (33 28′ N, 117 2′ W) at 500 m in elevation. Much of the Reserve burned in a wildfire in 2004 and we sampled in areas where chaparral had recovered, and areas where exotic grasses persisted. We collected soil inoculum from underneath *Bromus diandrus* (n = 15) in a heavily invaded area and underneath *Adenostoma fasciculatum* (n = 15) in an adjacent area of mature chaparral. Soil characteristics for native and invasive soils can be found in Table S2. For inoculation, we did not pool replicate samples and instead inoculated each of 15 pots per treatment with each individual replicate separately. We collected *A. fasciculatum* seeds at Emerson Oaks Reserve from ten mature individuals adjacent to an area invaded by *Bromus diandrus* and mixed them. They were stored at room temperature for 2–3 months. Prior to planting we scarified them in a 10% sulfuric acid solution for ten minutes. For the potting mix, we collected soil from five locations at the native-invasive vegetation interface, composited this soil and diluted it 50% with silica sand to improve drainage, a common practice for inoculum studies in fine-textured soil (e.g., Johnson et al., 2008). We steam- sterilized at 100 °C this field soil – sand mixture for 24 h, held at room temperature for 24 h, and steam-sterilized for another 24 h. We placed the soil into sterilized 800 ml Conetainer® pots and mixed with 40 g of one of the following field-collected soil inoculum treatments: native (collected under *A. fasciculatum*, n = 15), invasive (collected under *B. diandrus*, n = 15), and sterile (20 g from sterilized invasive and 20 g sterilized native collected field soil). We germinated seeds in these Conetainers, thinned to one individual per plot, and harvested at six months. We made efforts to minimize contamination by keeping inoculum treatments separate from one another, while keeping conditions consistent between treatments by rotating pots biweekly. While we made efforts to minimize contamination by using a climate-controlled greenhouse, sterile conditions could not be maintained in the shared space, thus making airborne contamination unavoidable.

2.2. Percent colonization

At the time of harvest, we rinsed 0.15 g of fresh roots with DI water and reserved them for DNA extraction by storing them in a −20 °C freezer. We weighed the remainder of the fresh roots for calculating water content of the roots to account for the fresh roots removed for molecular analyses. We placed fresh roots and shoots in coin envelopes, dried them at 60 °C for 48 h, and weighed them to determine seedling biomass. We rehydrated the dried root biomass to examine mycorrhizal colonization. We cleared roots 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 plant were observed at 200× magnification. We examined root fragments for AMF hyphae, arbuscules, vesicles, as well as for non-AM fungal hyphae. We also assessed *A. fasciculatum* roots for ectomycorrhizal fungal (EMF) mantles and Hartig nets at 50× magnification as this species is known to associate with EMF in moist soils (Allen et al., 1999).

2.3. Library construction and sequencing

We extracted DNA from 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 twenty minutes, before homogenization (Rubin et al., 2014). Samples were kept frozen in a −20 °C freezer 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 & Reich 2012. DNA concentrations were determined by PicoGreen (Molecular Probes Inc., Eugene OR, USA) fluorescence and subsequently standardized each sample to ~10 ng/μL.

We amplified samples by polymerase chain reaction (PCR) for the 18S 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. (2012). First-round amplifications were carried out 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. We checked the results of the reaction products on a 1% agarose gel. We purified products using a PEG-bead cleanup and eluted in 20 μL Tris-Cl (pH 8.0); we combined 1 μL of purified sample with 9 μL of Tris-Cl (pH 8.0), and used diluted product 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 reaction 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 checked indexed PCR products on an agarose gel, and then purified the products with the PEG-bead cleanup, quantified by PicoGreen fluorescence, and pooled equimolar concentrations of each sample combining them 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.

2.4. Bioinformatics

We joined forward and reverse reads for the ITS2 locus using multiple_join_paired_ends.py in QIIME 1.9.1 (Caporaso et al., 2010) allowing 30% max differences and a minimum overlap of 30. For the 18S locus, we used only the forward read. Demultiplexing and quality filtering was carried out using multiple_split_libraries_fastq.py in QIIME 1.9.1 (Caporaso et al., 2010) with the command options q = 19, r = 0 and p = 0.95. We removed chimeras with VSEARCH (Rognes et al., 2016) using the uchime_de-novo option for 18S or using the -uchime_ref option against the UNITE fungal chimera reference for ITS2 (Nilsson et al., 2015). We extracted fungal sequences from the ITS2 locus using ITSx (Bengtsson-Palme et al., 2013). We picked OTUs using swarm with a resolution of *d4*, which collapses sequences with less than 4 differences into a single representative OTU. We assigned taxonomy using BLAST at 97% similarity against the UNITE database (Kõljalg et al., 2013) for ITS2 and MaarjAM database for 18S (Öpik et al., 2010). OTUs comprising less than 0.005% of the total dataset were removed (Bokulich et al., 2013). OTU tables were rarefied to 14,370 reads for ITS2 and 7386 reads for 18S for alpha diversity analyses. We normalized OTU tables using cumulative sum scaling (CSS) normalization in the metagenomeSeq package of Bioconductor (Paulson et al., 2013) for all other downstream analyses. Raw and CSS-normalized OTU tables are available through Mendeley Data at https://doi.org/10.17632/gktc62bnhj.1 (Phillips, 2019).

2.5. 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 18S sequences. The remaining non-AMF symbionts includes EMF. 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. To interpret responses of the AMF community (18S) we assigned families of Glomeromycotina to AMF functional groups: rhizophilic, edaphophilic and ancestral using AMF resource allocation patterns defined in previous studies (Table S1, Weber et al., 2019; Phillips et al., 2019).

2.6. Statistical analyses

We used the root and shoot biomass (g) data to calculate root to shoot ratios. We fit linear models using 'lm' function from the 'stats' package in R where root:shoot, root biomass, or shoot biomass were the response variables and inoculum source was the predictor variable. We used an ANOVA and a Tukey's Honest Significant Differences post-hoc test to determine if there were significant differences in root:shoot ratios, root biomass, or shoot biomass between inoculum sources. We used non-parametric Kruskal-Wallis rank sum tests when the response variable was not normally distributed. To evaluate whether soil inoculum source affected root and shoot biomass, we used the 'kruskal.test' function from the 'stats' package followed by the 'dunnTest' function from the 'FSA' package (Ogle, 2018) with the bonferroni method to control the experiment-wise error rate. To test for differences in AMF and non-AMF colonization between roots grown with each inoculum source, we used a Kruskal-Wallis rank sum test ('kruskal.test' function from the 'stats' package in R). If the Kruskal-Wallis test was significant we used the 'dunnTest' function from the 'FSA' package (Ogle, 2018) with the bonferroni method to examine pairwise comparisons of inoculum source (native, invasive and sterile).

We calculated the alpha diversity for each sample by both ITS2 and 18S loci (Chao1, Shannon, and Observed Species) using the core_diversity_analyses.py function in QIIME 1.9.1 (Caporaso et al., 2010). We used the 'kruskal.test' function to determine if there were significant differences in diversity between inoculum source and if significant, we tested the significance of pairwise comparisons using the 'dunnTest' function from the 'FSA' package (Ogle, 2018). 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. The fit of the data was assessed via the stress values associated with the NMDS, with stress values of less than 0.2 deemed acceptable. We tested for differences between inoculum sources in overall general fungal (ITS2) and AMF (18S) community composition across inocula sources by performing permutational multivariate ANOVA (PERMANOVA) for each locus using the 'adonis' function in the 'vegan' package in R (999 permutations; Oksanen et al., 2017). Additionally, we evaluated differences in the OTU richness of the functional groups for both ITS2 and 18S loci, as described above using the Kruskal-Wallis rank sum test and Dunn's Kruskal-Wallis Multiple Comparisons. All statistical analyses were performed in R version 3.4.4 (R version 3.4.4; R Core Team 2018).

2.7. Indicator species analysis

We tested for indicator species/taxa associated with each of the different inoculum treatments for the ITS2 locus, with a particular interest in EM species. We calculated the indicator values using the 'multiplatt' function with 9999 permutations in the 'indicspecies' R package (Cáceres and Legendre 2009). Indicator value indices are used for assessing the predictive values of species as indicators of conditions present in the different groups (Cáceres and Legendre 2009). We only retained taxa with a p value < 0.05 as significant.

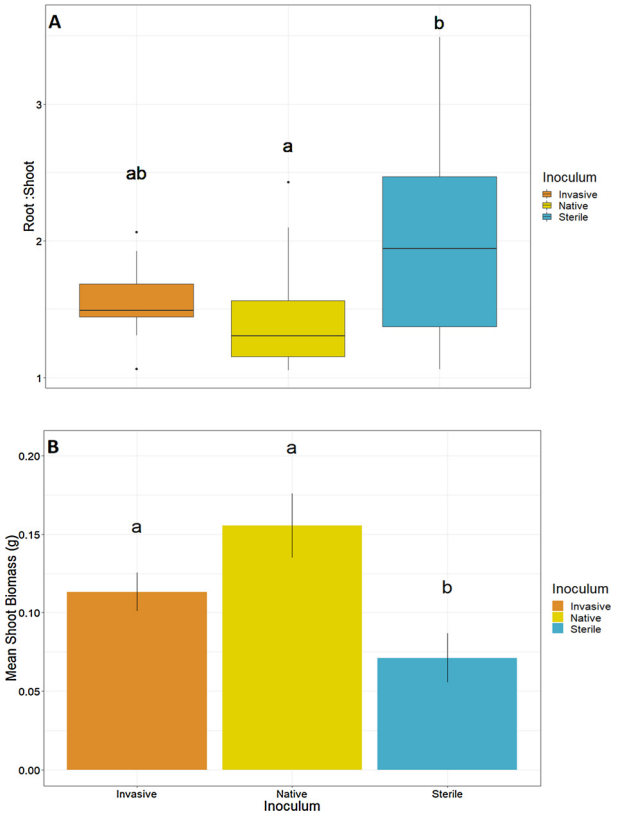

Fig. 1. (A) Root to shoot ratio and **(B)** mean shoot biomass (g) of *Adentostoma fasciculatum* seedlings grown with three inoculum types (n = 15) at harvest. Letters indicate significant differences at $p < 0.05$.

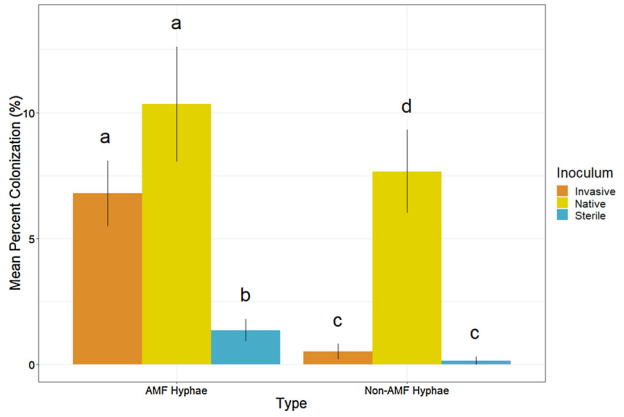

Fig. 2. Percent colonization of *Adenostoma fasciculatum* roots grown with three inoculum types (n = 15). Letters indicate significant differences at $p < 0.05$.

3. Results

3.1. Growth response

Adenostoma fasciculatum seedlings grown in either native or invasive inocula had equivalent root:shoot ratios (p = 0.85, Fig. 1A, Table S3). Seedlings grown with native inoculum had a significantly lower root:-shoot ratio than those grown with sterile inoculum (p = 0.02, Fig. 1A, Table S3). *Adenostoma fasciculatum* seedlings grown with native inoculum produced neither more root nor shoot biomass than those grown with invasive inoculum (Figure S1 and 1B, p = 0.24 and 0.70, respectively). However, seedlings grown with both native and invasive inocula produced both more root (Figure S1, p = 0.02 and 0.03, respectively; Table S3) and shoot biomass (Fig. 1B, p = 0.002 and 0.05, respectively; Table S3) than those grown with sterile inoculum.

3.2. AMF colonization, composition and richness

3.2.1. Percent colonization

Roots of *A. fasciculatum* grown with native inoculum had higher rates of AMF colonization (10% ± 2.3 (mean ± SE)) than those grown with sterile inoculum (1.4% ± 0.44), but not higher than those grown with invasive inoculum (7% ± 1.3; P = 0.001 and 0.8, respectively; Fig. 2, Table S4). Seedlings grown with native inoculum also had higher rates of non-AMF colonization (8% ± 1.7) than those grown with either sterile (0.15% ± 0.15; P = 0.0003) or invasive inocula sources (0.51% ± 0.29; P = 0.002, Fig. 2, Table S4).

3.2.2. 18S sequences

We observed a total of 234 OTUs that were assigned to known taxa after performing BLAST against the MaarjAM database. After CSS-normalization, we observed a mean of 592 ± 14 (SE) reads, and 120 ± 3 OTUs, per sample. These OTUs belonged to 4 orders, 9 families and 8 genera within Glomeromycotina. We observed the following 8 genera: *Glomus, Acaulospora, Archaeospora, Paraglomus, Scutellospora, Claroideoglomus, Ambispora,* and *Diversispora*. Roots grown with native inoculum had 84 ± 10 reads and 16 ± 2 OTUs per sample. Roots grown with invasive inoculum had 73 ± 10 reads and 14 ± 2 OTUs per sample and roots grown with sterile inoculum had 63 ± 10 reads and 13 ± 2 OTUs per sample. We placed these OTUs into three functional guilds, as described previously (Table S1). Of these functional guilds, the most common guild was rhizophilic AMF families (546 ± 12 reads and 112 ± 2 OTUs per sample), followed by ancestral families (50 ± 0.5 reads and 37 ± 3 OTUs per sample), with edaphophilic AMF families being the least common (8 ± 1 reads and 1 ± 0.05 OTUs per sample) functional guild detected in our study.

3.2.3. AMF community composition and richness

We only observed a difference in Shannon diversity between roots grown with invasive and native inocula sources (P = 0.002; Table S5) and sterile and native inocula (P = 0.002; Table S5). Roots grown with native inoculum had higher Shannon diversity values for AMF than those grown with either invasive and sterile inocula sources. We did not detect any differences in beta diversity of the AMF (18S) community (P = 0.09, Fig. 3).

Roots grown with native inoculum hosted a richer community of rhizophilic AMF – families that primarily produce intraradical hyphae – than both those grown in invasive and sterile inocula (P = 0.37 and 0.003, respectively; Table S6, Fig. 4). There was no difference in the richness of rhizophillic AMF between roots grown with invasive and

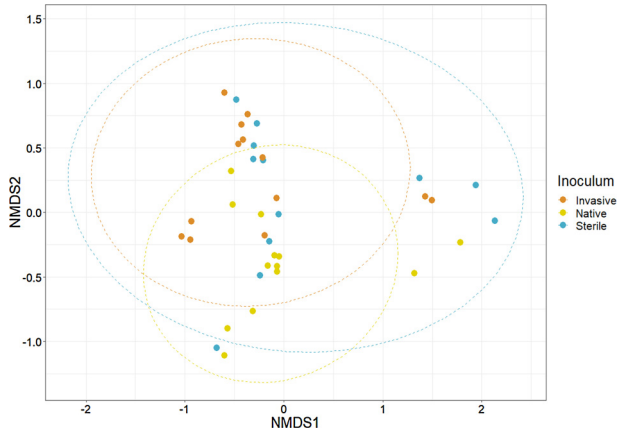

Fig. 3. Arbuscular mycorrhizal fungi (AMF; 18S) Bray-Curtis NMDS plot. Color is inoculum type (native, invasive, or sterile). The stress value is 0.13.

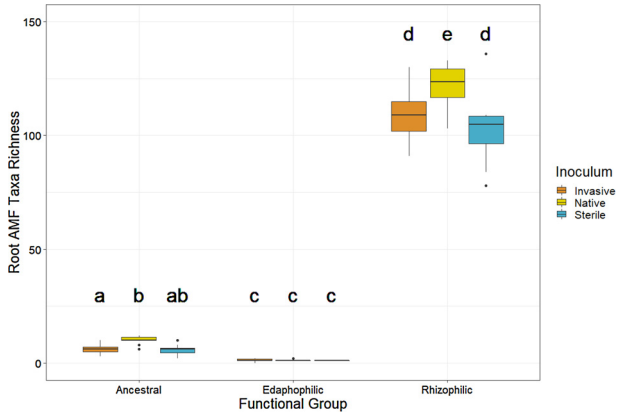

Fig. 4. 18S or arbuscular mycorrhizal fungal (AMF) root community by functional group. AMF taxa richness is the number of times a unique taxonomic unit is encountered in each sample Letters indicate significant differences at $p < 0.05$ and can be found in in Table S4.

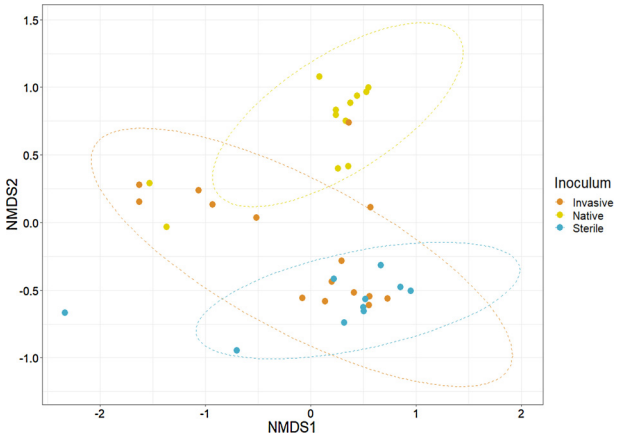

Fig. 5. General Fungal Community (ITS2) Bray-Curtis NMDS plot. Color is inoculum type (native, invasive, or sterile). The stress value is 0.066.

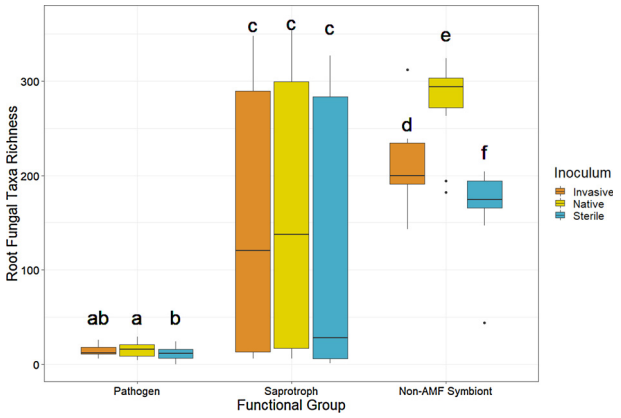

Fig. 6. ITS2 or general fungal community root community by functional group by aggregating species using FUNguild. Fungal taxa richness is the number of times a unique taxonomic unit is encountered in each sample. Letters indicate significant differences at $p < 0.05$ and can be found in Table S4.

sterile inocula (P = 0.94; Table S6; Fig. 4). There were no differences in richness of edaphophilic AMF families that primarily produce extraradical or foraging hyphae between inoculum sources (P = 0.91, 0.52 and 0.99; Table S6; Fig. 4). Roots grown with native inoculum hosted a richer community of ancestral AMF than both invasive and sterile inoculum sources (P = 0.001 and 0.001, respectively; Table S6; Fig. 4).

3.3. General fungal community composition and richness

3.3.1. ITS2 sequences

After CSS-normalization, we observed a mean ± SE of 2464 ± 62 reads and 738 ± 25 OTUs per sample. These OTUs belonged to 6 phyla, 15 classes, 40 orders, 68 families and 116 genera. Roots grown with native inoculum had 23 ± 2 reads and 7 ± 1 OTUs per sample. Roots grown with invasive inoculum had 22 ± 2 reads and 7 ± 1 OTUs per sample and roots grown with sterile inoculum had 18 ± 2 reads and 5 ± 1 OTUs per sample. The most abundant phylum in the roots was Ascomycota with 2225 ± 58 reads and 667± 22 OTUs per sample, followed by Basidiomycota with 155 ± 13 reads and 42 ± 2 OTUs. Symbiotic fungi were most common (863 ± 59 reads and 221 ± 10 OTUs per sample), followed by saprotrophic fungi (481 ± 56 reads and 149 ± 16 OTUs per sample) and fungal pathogens (44 ± 2 reads and 14 ± 0.6 OTUs per sample). Once we removed AMF from our analyses, to account for any overlap between our 18S and ITS2 datasets, the remaining fungal symbionts consisted of 65 families, 111 genera, and 243 species; these symbionts consisted of eleven families – Tuberaceae, Pyronemataceae, Atheliaceae, Tricholomataceae, Thelephoraceae, Pezizaceae, Discinaceae, Rhizopogonaceae, Hygrophoraceae – which contain ectomycorrhizal taxa.

3.3.2. General fungal community composition and richness

We detected higher alpha diversity values– Shannon, chao1, and observed species – in roots grown with both native (P = 0.01, 0.0002 and 0.0002, respectively; Table S5) and invasive (P = 0.03, 0.01 and 0.01, respectively; Table S5) inocula, than were detected in roots grown with sterile inoculum. There was no difference in alpha diversity values – Shannon, chao1, and observed species – for the ITS2 locus between roots grown with native and invasive inocula (P = 0.92, 0.81 and 0.53, respectively; Table S5). General fungal community (ITS2) beta diversity did not differ between inocula types (P = 0.9, Fig. 5).

Non-AMF symbionts (primarily ectomycorrhizae) had the highest species richness overall; roots grown with native inoculum hosted a richer community of non-AMF symbionts than those grown with either invasive or sterile inocula sources (P = 0.02 and 0.0001; Table S6; Fig. 6). Roots grown with invasive inoculum also hosted a richer community of non-AMF symbionts than those grown with sterile inoculum (P = 0.01; Table S6; Fig. 6). Although the pathogenic fungal community hosted by roots grown with native inoculum was richer than those grown with sterile inoculum (P = 0.04; Table S6; Fig. 6), we did not detect any differences in richness between roots grown with native or invasive inocula (P = 0.53; Table S6; Fig. 6). Additionally, there were no detectable differences in pathogen richness between communities hosted by roots grown with invasive and sterile inocula (P = 0.65; Table S6; Fig. 6). The richness of saprotrophs in roots did not differ between inocula (P = 0.42, 0.22 and 0.07; Table S6, Fig. 6).

3.3.3. Indicator species analysis

Indicator species analysis yielded a total of 99 significant taxa for all inoculum sources. Roots grown with native inoculum produced 75 significant taxa, followed by 63 significant taxa associated with invasive inoculum, and roots grown with sterile inoculum yielded 9 significant taxa (Fig. 7). There were 13 EM species (*Geopora cooperi*, *Choiromyces alveolatus*, *Choiromyces* sp, *Tylospora* sp PG, *Tomentella cinerascens*, *Tuber* sp, *Geopora* sp BS_2010, *Gilkeya compacta*, *Rhodoscypha* sp, *Tuber separans*, *Tuberaceae* sp, *Wilcoxina rehmii*) with significant indicator values associated with roots grown with native inoculum, followed by 5 EM species associated with invasive inoculum (Fig. 7, Table S7). Two EM species had significant indicator values associated with roots grown in sterile inoculum (Table S7). Additionally, the endophytes *Penicillium brevicompactum* and *Capronia* sp. had significant indicator values associated with native inoculum, whereas *Capronia* sp.

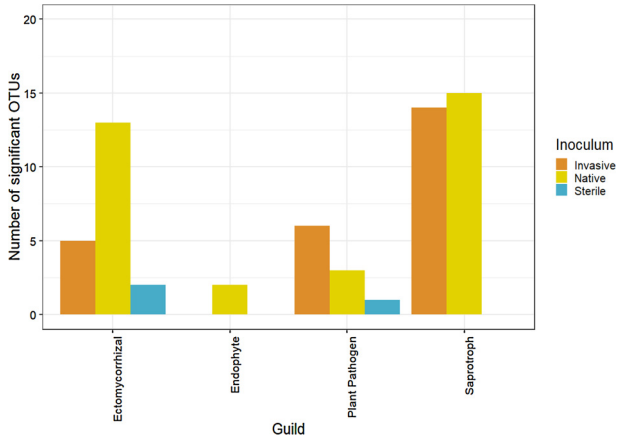

Fig. 7. Number of OTUs that were significant indicator species for each inoculum type with species grouped by Guilds assigned using FUNguild.

was the only endophyte species with a significant indicator value that was associated with invasive inoculum (Table S7). Three plant pathogen species (*Stagonospora perfecta*, *Lectera longa* and *Pseudofusicoccum kimberleyense*) had significant indicator values associated with native inoculum and six plant pathogen species (*Dothiorella brevicollis, Mastigosporium album*, *Powellomyces* sp, *Lectera longa, Pseudofusicoccum kimberleyense* and *Powellomyces hirtus*) had significant indicator values associated with invasive inoculum (Table S7). One species (*Mastigosporium album*) was a significant indicator value associated with sterile inoculum. Native and invasive inoculum sources had nearly the same amount of significant indicator species belonging to the saprotroph guild (14 and 15, respectively), whereas there were no significant species in the sterile inoculum that belonged to the saprotroph guild (Fig. 7, Table S7).

4. Discussion

Soil microbial communities play a key role in the development of soil health (Anderson, 2003) and have proven to be an important factor in contributing to the success of restoration efforts because of their ability to affect plant successional dynamics and resulting community composition. In the context of invasion, we know that invasive grasses can shift the composition of key soil microbial groups, such as fungal symbionts, thus creating novel soil microbial communities (Busby et al., 2013, 2011; Hausmann and Hawkes, 2009; Zhang et al., 2010). More specifically, some studies suggest that invasive annual grasses are less dependent on AMF mutualisms than the native species that previously occurred where they have invaded (Allen, 1984; Richardson et al., 2000; Callaway et al., 2004; Reinhart and Callaway, 2006; Busby et al., 2011, 2013). According to the degraded mutualist hypothesis (Vogelsang and Bever, 2009), if these invasive grasses are less dependent on AMF then we may expect overall fewer plant species dependent on mutualisms within the vegetation community. Our results show some support for the degraded mutualist hypothesis, such that invasive-conditioned soils can decrease the richness and abundance of soil symbionts. Likewise, we found that seedlings grown with native inoculum hosted richer communities of both rhizophilic and ancestral AMF, as well as non-AMF symbionts, than those grown with invasive inoculum (Phillips et al., 2019; Busby et al., 2013, 2011; Hawkes et al., 2006). Yet, contrary to the degraded mutualist hypothesis, we did not detect any differences in AMF colonization between native and invasive colonization.

In addition to hosting a richer community of soil symbionts, we detected more species of EM fungi associated with native inoculum than with invasive inoculum. Although EM taxa may provide benefits to *A. fasciculatum* in soils conditioned by native plants, these EM fungi may be less prevalent in invasive-conditioned soils. A previous study has shown that our focal species, *A. fasciculatum*, can make associations with both AMF as well as EM fungi under moist conditions (Allen et al., 1999), which is likely a driver of the higher EM richness we observed in roots grown with native inoculum. Additionally, we observed lower richness of EM in invasive inoculum, suggesting that invasive grass encroachment may decrease EM colonization perhaps by depleting soil moisture (Melgoza et al., 1990). The presence of a diverse suite of EM taxa may aid in the restoration of slow-growing chaparral shrubs, like *A. fasciculatum*. Previous studies suggest that slow-growing shrubs are more likely to be dependent on locally adapted symbiotic associations for establishment (Azcón-Aguilar et al., 2003; Johnson et al., 2009).

We did not detect any differences in our beta diversity analyses between either the general fungal or AMF communities by inoculum source, likely because inoculum source did not influence edaphophilic AMF and saprotroph richness. We observed low richness values of edaphophilic AMF across all inoculum sources which is likely because we conducted our study in a greenhouse and families within these groups primarily produce extraradical or foraging hyphae that may provide less benefits when grown in a pots (Powell et al., 2009; Hart and Reader, 2002; Varela-Cervero et al., 2016b). However, while we observed low richness of edaphophilic AMF families in roots across inoculum sources, fungal spores may be present within the inoculum; the dormant spores have the potential to colonize roots when transplanted to the field and then aid in resource acquisition.

Our results suggest that native inoculation increases the richness of rhizophilic AMF, consisting of families that primarily colonize roots internally with lower allocation to extraradical hyphae, and provide protection from pathogenic fungi (Maherali and Klironomos, 2007; Sikes et al., 2009). Our findings suggest that *A. fasciculatum* seedlings may be more susceptible to pathogens in native soils, meaning that chaparral seedlings may be susceptible to the same pool of fungal pathogens regardless of inoculum source. However, it is likely that native seedlings will not have the same level of pathogen protection when grown in invasive-conditioned soils.

While seedlings grown with native inoculum hosted more symbionts overall, there were no differences in biomass produced between seedlings grown with native and invasive inoculum sources. Other studies have observed that invasive inoculum may promote growth more than native inoculum (Gillespie and Allen, 2006) or vice-versa (Wubs et al., 2006; Middleton and Bever, 2012); these responses may not only be site specific but also likely related to the microbial community and their host plants (Eviner and Hawkes, 2008). It is worth noting that our focal plant, *Adenostoma fasciculatum* is a slow growing species; therefore, detectable differences in *A. fasciculatum* biomass may develop over a longer duration of time than within a six-month growing period.

A richer and more abundant community of symbionts has the potential to aid in plant establishment when seedlings are out-planted in the field (Allen et al., 2003, 2005). Other studies have demonstrated that native inoculation can increase the establishment of native plant species (Requena et al., 2001; Wubs et al., 2016; Middleton and Bever, 2012). Although we expected that seedlings grown with native inoculum would produce more biomass than those grown with sterile and invasive inocula, we only found partial support for this hypothesis; seedlings grown with both native and invasive inocula produced more biomass than those grown with sterile inoculum. Interestingly, seedlings grown with sterile inoculum had a higher root to shoot ratio which may result from the low taxa richness and abundance of symbionts causing seedlings to invest more in belowground biomass. Furthermore, a diverse assemblage of symbionts in inoculated treatments likely contributed to higher root and shoot biomass of seedlings grown with invasive and native inoculum than those grown with sterile inoculum. This suggests that inoculation, from any inoculum source, provides benefits for seedlings propagated in a nursery intended for out-planting in the field, and may assist practitioners in achieving successful restoration outcomes.

While consideration of microbial community composition, and particularly mycorrhizal symbionts, have become more integrated into restoration ecology (Wubs et al., 2016; Maltz and Treseder, 2015; Middleton and Bever, 2012; Requena et al., 2001), generalizing this knowledge for chaparral restoration has proven challenging (Allen et al., 2018). Chaparral requires active restoration efforts, as well as careful consideration when selecting species to cultivate in the nursery for restoration efforts, because there has been little documented success in chaparral plant establishment via broadcasting seed. Although we selected *A. fasciculatum*, we acknowledge that, in certain circumstances, it may be more practical to use a faster-growing species that can be transplanted to the field more rapidly. Findings from our study suggests that efforts aimed at growing chaparral seeds collected from local populations within nursery environments, combined with native-conditioned inoculum, may improve out-planting success at candidate sites (Allen et al., 2018; Stratton, 2005). Moreover, out-planting seedlings grown with native inoculum may not only promote a diverse community of soil symbionts but may also lead to greater abundance within multiple groups of soil symbionts.

Increasing the abundance and richness of soil symbionts has implications for restoration. For instance, diverse microbial communities may increase interactions between host-plants and soil symbionts. Additionally, a thriving resident soil microbial community, replete with chaparral symbionts, has the potential to heighten the viability of hitherto unsuccessful broadcast seeding techniques. Future research should assess if out-planting native seedlings cultivated together with native inoculation improves revegetation success, as these seedlings could serve as nurse plants by creating more favorable microbial communities (Azcón-Aguilar et al., 2003).

5. Conclusions

Soil inoculum affected the community of both AMF and non-AMF symbionts that colonized the roots of *A. fasciculatum* seedlings. Seedlings grown with native inoculum hosted a richer community of fungal symbionts than those grown with invasive and sterile inoculum, suggesting that invasive conditioned soils may reduce the presence of symbiotic fungi (Hawkes et al., 2006; Busby et al., 2011, 2013). Yet, we detected higher rates of AMF and non-AMF colonization in roots grown with native inoculum than those grown with sterile, but not invasive inoculum. Our greenhouse study does not preclude previous findings that native inoculum may increase establishment when transplanted to type-converted field sites (Middleton and Bever, 2012; Wubs et al., 2016). We conclude that inoculating the soil with live soil inoculum, invasive or native, led to both a more diverse fungal community and a positive plant growth response. Future research would benefit from including a diverse assemblage of focal species to investigate the effects of soil inoculation on chaparral growth responses. Overall, our results demonstrate the importance of including soil inoculation along with active restoration techniques when propagating chaparral shrubs to support successful restoration efforts.

Declaration of Competing Interest

The authors have no conflicts of interest to declare.

Acknowledgements

We thank Alexandra Johnson, Sameer Saroa, Cierra Wikman and Aleric Krenz who aided in the propagation of plants and harvesting our greenhouse experiment. Funding was provided by University of California Riverside, Department of Botany and Plant Sciences and the Center for Conservation Biology, as well as a graduate fellowship from NASA MUREP Institutional Research Opportunity (MIRO) grant number NNX15AP99A, and a graduate fellowship from the University of California's Institute for the Study of Ecological and Evolutionary Climate Impacts (ISEECI), funded by a UC Presidential Research Catalyst Award.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.apsoil.2019.103370.

References

Allen, E.B., 1984. Mycorrhizae and colonizing annuals: implications for growth, competition, and succession. In: Williams, S.E., Allen, M.F. (Eds.), VA Mycorrhizae and Reclamation of Arid and Semiarid Lands. University of Wyoming Agr. Expt. Sta. Laramie, Wyoming, pp. 42–52.

Allen, E.B., Allen, M.F.A., Corkidi, L., Egerton-warburton, L., Gomez Pompa, A., 2003. Impacts of early- and late-seral mycorrhizae during restoration in seasonal tropical forest, Mexico. Ecol. Appl. 13, 1701–1717.

Allen, M.F., Allen, E.B., Gomez-Pompa, A., 2005. Effects of mycorrhizae and nontarget organisms on restoration of a seasonal tropical forest in Quintana Roo, Mexico: factors limiting tree establishment. Restor. Ecol. 13, 325–333.

Allen, M.F., Egerton-Warburton, L.M., Allen, E.B., Karen, O., 1999. Mycorrhizae in *Adenostoma fasciculatum* Hook. And Arn.: a combination of unusual ecto- and endoforms. Mycorrhiza 8, 225–228.

Allen, E.B., Williams, K., Beyers, J.L., Phillips, M.L., Ma, S., D'Antonio, C.M., 2018. Chaparral Restoration. Pages 347-384 In: Underwood, E., Safford, H., Molinari, N., Keeley, J. (Eds.), Valuing Chaparral, Ecological, Socio-Economic, and Management Perspectives. Springer International Publishing, pp. 2019.

Alvarado, P., Teixeira, M., Andrews, L., Fernandez, A., Santander, G., Doyle, A.L., Perez, M., Yegres, F., Mendoza, M., Barker, B.M., 2018. Molecular detection of Coccidioides posadasii and associated fungal communities from soils in endemic areas of coccidioidomycosis in Venezuela. Emerg. Microbes Infect. 7 (46).

Anderson, T., 2003. Microbial eco-physiological indicators to asses soil quality. Agric., Ecosyst. Environ., Appl. Soil Ecol. 98, 285–293.

Azcón-Aguilar, C., Palenzuela, J.R., Bautista, S., Vallejo, R., Barea, J.M., 2003. Analysis of the mycorrhizal potential in the rhizosphere of representative plant species from desertification-threatened Mediterranean shrublands. Appl. Soil Ecol. 22 (1), 29–37.

Bengtsson-Palme, J., Ryberg, M., Hartmann, M., Branco, S., Wang, Z., Godhe, A., Wit, P., De Marisol, S., Ebersberger, I., Sousa, F., De Amend, A.S., Jumpponen, A., Unterseher, M., Kristiansson, E., Abarenkov, K., Bertrand, Y.J.K., Sanli, K., Eriksson, K.M., Vik, U., Veldre, V., Nilsson, R.H., 2013. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. Methods Ecol. Evol. 4, 914–919.

Berry, D., Ben Mahfoudh, K., Wagner, M., Loy, A., 2012. Barcoded primers used in multiplex amplicon pyrosequencing bias amplification. Appl. Environ. Microbiol. 78, 612.

Bokulich, N.A., Subramanian, S., Faith, J.J., Gevers, D., Gordon, J.I., Knight, R., Mills, D.A., Caporaso, J.G., 2013. Quality filtering vastly improves diversity estimates from Illumina amplicon sequencing. Nat. Methods 10, 57–59.

Busby, R.R., Gebhart, D.L., Stromberger, M.E., Meiman, P.J., Paschke, M.W., 2011. Early seral plant species' interactions with an arbuscular mycorrhizal fungi community are highly variable. Appl. Soil Ecol. 48, 257–262.

Busby, R.R., Stromberger, M.E., Rodriguez, G., Gebhart, D.L., Paschke, M.W., 2013. Arbuscular mycorrhizal fungal community differs between a coexisting native shrub and introduced annual grass. Mycorrhiza 23, 129–141.

Callaway, R.M., Cipollini, D., Barto, K., Thelen, G.C., Hallett, S.G., Prati, D., Stinson, K., Klironomos, J., 2008. Novel weapons: invasive plant suppresses fungal mutualists in America but not in its native Europe. Ecology 89, 1043–1055.

Callaway, R.M., Thelen, G.C., Barth, S., Ramsey, P.W., Gannon, J.E., 2004. Soil Fungi alter interactions between the invader *Centauria maculosa* and North American Natives. Ecology 85, 1062–1071.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., Mcdonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Turnbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data Intensity normalization improves color calling in SOLiD sequencing. Nat. Publ. Group 7, 335–336.

Chen, H.Y.H., Brassard, B.W., 2013. Intrinsic and extrinsic controls of fine root life span. Crit. Rev. Plant Sci. 32, 151–161.

D'Antonio, C.M., Vitousek, P.M., 1992. Biological invasions by exotic grasses, the Grass/Fire cycle, and global change. Annu. Rev. Ecol. Syst. 23, 63–87.

Dickens, S.J.M., Allen, E.B., 2014. Exotic plant invasion alters chaparral ecosystem resistance and resilience pre- and post-wildfire. Biol. Invasions 16 (5), 1119–1130.

Dumbrell, A.J., Ashton, P.D., Aziz, N., Feng, G., Nelson, M., Dytham, C., Fitter, A.H., Helgason, T., 2011. Distinct seasonal assemblages of arbuscular mycorrhizal fungi revealed by massively parallel pyrosequencing. New Phytol. 190, 794–804.

Eviner, V.T., Hawkes, C.V., 2008. Embracing variability in the application of plant-soil interactions to the restoration of communities and ecosystems. Restor. Ecol. 4 (16), 713–729.

Gillespie, I.G., Allen, E.B., 2006. Effects of soil type and mycorrhizae from native and invaded vegetation on a rare California forb. Appl. Soil Ecol. 32, 6–12.

Hart, M.M., Reader, R.J., 2002. Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. New Phytol. 153, 335–344.

Hausmann, N.T., Hawkes, C.V., 2009. Plant neighborhood control of arbuscular mycorrhizal community composition. New Phytol. 183, 1188–1200.
Hawkes, C.V., Belnap, J., D'Antonio, C., Firestone, M.K., 2006. Arbuscular mycorrhizal assemblages in native plant roots change in the presence of invasive exotic grasses. Plant Soil 281 (1–2), 69–380.
Hilbig, B.E., Allen, E.B., 2015. Plant-soil feedbacks and competitive interactions between invasive *Bromus diandrus* and native forb species. Plant Soil 392, 191–203.
Inderjit, van der Putten, W.H., 2010. Impacts of soil microbial communities on exotic plant invasions. Trends Ecol. Evol. (Amst.) 25 (9), 512–519.
Johnson, N.C., Rowland, D.L., Corkidi, L., Allen, E.B., 2008. Characteristics of plant winners and losers during grassl and eutrophication – importance of biomass allocation and mycorrhizal function. Ecology 89, 2868–2878.
Johnson, N.C., Wilson, G.W.T., Bowker, M.A., Wilson, J.A., Miller, R.M., 2009. Resource limitation is a driver of local adaptation in mycorrhizal symbioses. Proc. Natl. Acad. Sci. U.S.A. 107, 2093–2098.
Keeley, J.E., Brennan, T.J., 2012. Fire-driven alien invasion in a fire-adapted ecosystem. Oecologia 169 (4), 1043–1052.
Kõljalg, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L., Taylor, A.F.S., Bahram, M., Bates, S.T., Bruns, T.D., Bengtsson-Palme, J., Callaghan, T.M., Douglas, B., Drenkhan, T., Eberhardt, U., Dueñas, M., Grebenc, T., Griffith, G.W., Hartmann, M., Kirk, P.M., Kohout, P., Larsson, E., Lindahl, B.D., Lücking, R., Martín, M.P., Matheny, P.B., Nguyen, N.H., Niskanen, T., Oja, J., Peay, K.G., Peintner, U., Peterson, M., Põldmaa, K., Saag, L., Saar, I., Schüßler, A., Scott, J., Senés, C., Smith, M.E., Suija, A., Taylor, D.L., Telleria, M.T., Weiss, M., Larsson, K.H., 2013. Towards a unified paradigm for sequence-based identification of fungi. Mol. Ecol. 22, 5271–5277.
Kormanik, P.P., McGraw, A.C., 1982. Quantification of vesicular-arbuscular mycorrhizas in plant roots. In: Schenck, N.C. (Ed.), Methods and Principles of Mycorrhizal Research. The American Phytopathological Society, St Paul, Minnesota, pp. 37–45.
Koske, R.E., Gemma, J.N., 1989. A modified procedure for staining roots to detect VA mycorrhizas. Mycol. Res. 92, 486–488.
Lee, J., Lee, S., Young, J.P.W., 2008. Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. FEMS Microbiol. Ecol. 65, 339–349.
Maherali, H., Klironomos, J.N., 2007. Influence of phylogeny on fungal community assembly and ecosystem functioning. Science (New York, N.Y.) 316, 1746–1748.
Maltz, M.R., Treseder, K.K., 2015. Sources of inocula influence mycorrhizal colonization of plants in restoration projects: a meta-analysis. Restor. Ecol. 23 (5) 635-634.
McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L., Swan, J.A., 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol. 115 (3), 495–501.
Melgoza, G., Nowak, R.S., Tausch, R.J., 1990. Soil water exploitation after fire: competition between *Bromus tectorum* (cheatgrass) and two native species. Oecologia 83, 7–13.
Meng, R., Dennison, P.E., D'Antonio, C.M., Moritz, M.A., 2014. Remote sensing analysis of vegetation recovery following short-interval fires in Southern California shrublands. PLoS One 9 (10), 14–17.
Middleton, E.J., Bever, J.D., 2012. Inoculation with a native soil community advances succession in a grassland restoration. Restor. Ecol. 20 (2), 218–226.
Minnich, R.A., Bahr, C.J., 1995. Wildland fire and chaparral succession along the California baja-California boundary. Int. J. Wildland Fire 5, 13–24.
Nelson, L.L., Allen, E.B., 1993. Restoration of *Stipa pulchra* grasslands: effects of mycorrhizae and competition from *Avena barbata*. Restor. Ecol. 1, 40–50.
Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., Schilling, J.S., Kennedy, P.G., 2016. FUNGuild : an open annotation tool for parsing fungal community datasets by ecological guild. Fungal Ecol. 20, 241–248.
Nilsson, R.H., Tedersoo, L., Ryberg, M., Kristiansson, E., Hartmann, M., Unterserher, M., Porter, T., Bengtsson-Palme, J., Walker, D., De Sousa, F., Gamper, H.A., Larsson, E., Larsson, K.H., Koljalg, U., Edgar, R.C., Abaren, 2015. A comprehensive, automatically updated fungal ITS sequence dataset for reference-based chimera control in environmental sequencing efforts. Microbes Environ. 30, 145–150.
Ogle, D.H., 2018. FSA: Fisheries Stock Analysis. R Package Version 0.8.21. https://github.com/droglenc/FSA.
Oksanen, J.F., Blanchet, G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P.M., Stevens, H.H., Szoecs, E., Wagner, H., 2017. Vegan: Community Ecology Package. R Package Version 2.4-3. https://CRAN.R-project.org/package=vegan.
Öpik, M., Vanatoa, a, Vanatoa, E., Moora, M., Davison, J., Kalwij, J.M., Reier, Ü., Zobel, M., 2010. The online database MaarjAM reveals global and ecosystemic distribution patterns in arbuscular mycorrhizal fungi (Glomeromycota). New Phytol. 188, 223–241.
Owen, S.M., Sieg, C.H., Johnson, N.C., Gehring, C.A., 2013. Exotic cheatgrass and loss of soil biota decrease the performance of a native grass. Biol. Invasions 15, 2503–2517.
Paulson, J.N., Stine, O.C., Bravo, H.C., Pop, M., 2013. Differential abundance analysis for microbial marker-gene surveys. Nat. Methods 10, 1200–1202.
Phillips, Michala, 2019. Native and invasive inoculation sources modify fungal community assembly and biomass production of a chaparral shrub. Mendeley Data v1.
Phillips, M.L., Weber, S.E., Andrews, L.V., Aronson, E.L., Allen, M.F., Allen, E.B., 2019. Fungal community assembly in soils and roots under plant invasion and nitrogen deposition. Fungal Ecol. https://doi.org/10.1016/j.funeco.2019.01.002.
Powell, J.R., Parrent, J.L., Hart, M.M., Klironomos, J.N., Rillig, M.C., Maherali, H., 2009. Phylogenetic trait conservatism and the evolution of functional trade-offs in arbuscular mycorrhizal fungi. Proc. R. Soc. B 4237–4245.
Pringle, A., Bever, J.D., Gardes, M., Parrent, J.L., Rillig, M.C., Klironomos, J.N., 2009. Mycorrhizal symbioses and plant invasions. Annu. Rev. Ecol. Evol. Syst. 40, 699–715.
Reinhart, K.O., Callaway, R.M., 2006. Soil biota and invasive plants. New Phytol. 170, 445–457.
Requena, N., Perez-Solis, E., Azcon-Aguilar, C., Jeffries, P., Barea, J.M., 2001. Management of indigenous plant-microbe symbioses aids restoration of desertified ecosystems. Appl. Environ. Microbiol. 67, 495–498.
Richardson, D.M., Allsopp, N., D'Antonio, C.M., Milton, S.J., Rejmánek, M., 2000. Plant invasions–the role of mutualisms. Biol. Rev. 75, 65–93.
Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH : a versatile open source tool for metagenomics. PeerJ 1–22.
Rubin, B.E., Sanders, J.G., Hampton-Marcell, J., Owens, S.M., Gilbert, J.A., Moreau, C.S., 2014. DNA extraction protocols cause differences in 16S rRNA amplicon sequencing efficiency but not in community profile composition or structure. Microbiology open 3, 910–921.
Sikes, B.A., Cottenie, K., Klironomos, J.N., 2009. Plant and fungal identity determines pathogen protection of plant roots by arbuscular mycorrhizas. J. Ecol. 97, 1274–1280.
Stratton, L.C., 2005. Santa Catalina Island experimental restoration trials: developing appropriate techniques. In: Garcelon, D.K., Schwemm, C.A. (Eds.), Pages, pp. 213–227 National Park Service Technical Publication CHIS-05–01. Institute for Wildlife Studies, Arcata, California, USA.
Stylinski, C.D., Allen, E.B., 1999. Lack of native species recovery following severe exotic disturbance in southern Californian shrublands. J. Appl. Ecol. 36 (4), 544–554.
Taylor, D.L., Walters, W.A., Lennon, N.J., Bochicchio, J., Krohn, A., Caporaso, J.G., Pennanen, T., 2016. Accurate estimation of fungal diversity and abundance through improved lineage-specific primers optimized for illumina amplicon sequencing. Appl. Environ. Microbiol. 82, 7217–7226.
Van Der Heijden, M.G.A., Bardgett, R.D., Van Straalen, N.M., 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol. Lett. 11 (3), 296–310.
van der Putten, W.H., Bardgett, R.D., Bever, J.D., Bezemer, T.M., Casper, B.B., Fukami, T., Kardol, P., Klironomos, J.N., Kulmatiski, A., Schweitzer, J.A., Suding, K.N., Van de Voorde, T.F.J., Wardle, D.A., 2013. Plant–soil feed- backs: the past, the present and future challenges. J. Ecol. 101, 265–276.
Varela-Cervero, S., López-García, Á., Barea, J.M., Azcón-Aguilar, C., 2016b. Differences in the composition of arbuscular mycorrhizal fungal communities promoted by different propagule forms from a Mediterranean shrubland. Mycorrhiza 489–496.
Vogelsang, K., Bever, J.D., 2009. Mycorrhizal densities decline in association with nonnative plants and contribute to plant invasion. Ecology 90, 399–407.
Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setala, H., van der Putten, W.H., Wall, D.H., 2004. Ecological linkages between aboveground and belowground biota. Science 304, 1629–1633.
Weber, S.E., Diez, J.M., Andrews, L.V., Goulden, M.L., Aronson, E.L., Allen, M.F., 2019. Responses of arbuscular mycorrhizal fungi to multiple coinciding global change drivers. Fungal Ecol In press.
Wickham, 2009. H. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.
Williamson, T.N., Newman, B.D., Graham, R.C., Shouse, P.J., 2004. Regolith water in zero-order chaparral and perennial grass watersheds four decades after vegetation conversion. Vadose Zone J. 3, 1007–1016.
Wolfe, B.E., Klironomos, J.N., 2005. Breaking new ground: soil communities and exotic plant invasion. Bioscience 55 (6), 477–487.
Wubs, E.R.J., van der Putten, W.H., Bosch, M., Bezemer, T.M., 2016. Soil inoculation steers restoration of terrestrial ecosystems. Nat. Plants 2 (8), 1–5.
Zhang, Q., Yang, R., Tang, J., Yang, H., Hu, S., Chen, X., 2010. Positive feedback between mycorrhizal Fungi and plants influences plant invasion success and resistance to invasion. PLoS One 5 (8).