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Invasion in the Chaparral: Uncovering Soil Microbial and Plant Physiological Mechanisms

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by

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ABSTRACT OF THE DISSERTATION

Invasion in the Chaparral: Uncovering Soil Microbial and Plant Physiological Mechanisms

by

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Doctor of Philosophy, Graduate Program in Plant Biology
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Dr. Edith Allen, Chairperson

Global change contributes to drastic shifts in vegetation composition resulting in changes in ecosystem processes across the world. One important example is plant invasion, which often leads to vegetation community type conversion, such as conversion from native shrubland to invasive grassland. Chaparral, California's most pervasive vegetation type, has recently undergone invasion. The shift from evergreen chaparral shrubs to invasive grassland will have cascading effects on ecosystem services. The overarching goal of this research is to understand water use, root and fungal dynamics of invaded chaparral communities that may inform restoration efforts. My first chapter examines how differences in root development relate to soil-water dynamics between a chaparral shrub and an invasive grass. I explored above- and below-ground strategies in concert of a native chaparral shrub and an invasive grass species in southern California using soil moisture sensors, manual minirhizotron imagery, stable isotopes, sap flux sensors and normalized difference vegetation index (NDVI). I found that the invasive grass species depleted soil moisture and produced longer roots earlier than the native shrub. Depletion of soil moisture earlier by *E. calycina* suggests that invasive grasses could accelerate the

onset of the summer drought in chaparral systems, assuring their persistence following invasion. My second chapter examines how invasion and nitrogen deposition structure composition of fungal communities. I found that invasive grasses had a lower richness and relative abundance of symbiotic fungi compared to native shrubs. My third chapter explores how invasive-conditioned soils affect the growth of chaparral shrub seedlings and associated fungal communities and I found that native inoculation produced a more diverse fungal symbiont community. Chapter four aims to detect if invasive grass water-use strategies are detrimental to shrub seedling success, and found that invasive removal positively affected the establishment of native shrub seedlings and seedling mortality, which increased alongside invasive cover. Overall, my dissertation demonstrates that competition between invasive and native plants as well as shifts in fungal communities contributes to invasive grass persistence and shows how joining tools and perspectives from diverse fields can provide a holistic look at system responses to change.

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Introduction

Global change contributes to drastic shifts in vegetation composition resulting in changes in ecosystem processes across the world (Walther et al. 2002; Gonzalez et al. 2010). One important example is terrestrial plant invasion by invasive species costing the world nearly $300 billion a year in damages and control cost alone (Pimental, 2002), while also having widespread effects on resource availability, disturbance regimes and other ecosystem functions (D'Antonio and Vitousek 1992). Invasion often leads to vegetation community type conversion, such as conversion from native shrubland to exotic grassland, which can be accompanied by changes in resource availability and soil microbial communities (Hawkes et al. 2005). Globally, Mediterranean ecosystems may be the one of the most severely affected by global change drivers (IPCC 2007). California recently experienced an extreme drought, a scenario that is expected to worsen as rainfall frequencies are projected to decrease (Sala et al. 2000). Southern California's systems are driven by intermittent resource pulses, and decreased rainfall coupled with increased inter-annual variability may create a more stochastic and unstable system in the future.

Chaparral was previously thought to be resilient to disturbance, yet has recently undergone invasion (Meng et al. 2014; Dickens and Allen 2014; Stylinski and Allen 1999; Keeley and Brennan 2012). The shift from evergreen chaparral shrubs to invasive grassland will have cascading effects on ecosystem services. Invasive species often have life history traits with flexible resource acquisition strategies (Ashbacher and Cleland 2015). Invasive annual grasses are drought escapers that may have short-lifespan, fine roots for rapid nutrient and water uptake. Alternatively, drought tolerant shrubs are

highly dependent on seasonal precipitation events for recharge through the soil profile (Schwinning and Ehleringer 2001), and are likely to possess long-lived relatively less efficient fine roots (Chen and Brassard 2013). These opposing life history traits are linked to differences in resource acquisition strategies that have the potential to affect soil water infiltration and reinforce the persistence of invasive grasses. Yet, efforts to restore native plant communities remain limited by our understanding of the mechanisms by which invasive plants outcompete native plants (Funk et al. 2016). To increase our ability to successfully restore native plant communities that provide essential ecosystem services, we need to mechanistically examine how ecological strategies of invasive plants allow them to persist and hamper restoration successes.

Invasion persistence is likely driven by multiple interacting mechanisms, such as the *a priori* presence of both mutualistic and parasitic soil microorganisms or alteration of the belowground community by the invasive species (Reinhart and Callaway 2006; Pringle et al. 2009; Van Der Heijden et al. 2008). When an invasive plant enters a native community, it alters aboveground inputs to the soil (e.g. decomposable litter, amount of photosynthates directed to mycorrhizae) which in turn may alter belowground community composition and function (Wolfe and Klironomos 2005; Inderjit and van der Putten 2010; Reinhart and Callaway 2006). There is substantial evidence that the enemy release hypothesis (invader success owing to reduced natural enemy attack) is an effective mechanism for the establishment of invasive weedy plants (Mitchell and Power 2003; Kardol et al. 2007; Reinhart et al. 2010; Van Grunsven et al. 2007).

One study reported that invasive neighbors (*Bromus hordeaceus* and *Avena barbata*) alter the community composition of arbuscular mycorrhizal fungi (AMF) found colonizing native roots, resulting in AMF community composition more similar to invasive associated communities relative to natives grown alone (Hawkes et al. 2006). This may be a result of invasive annuals' life history traits leading to earlier root activity than native perennial species (Hooper and Vitousek 1998), allowing for a 'priority effect' of invasive-associated AMF communities. Invasive grasses (e.g. *Bromus* spp.) may be facultatively mycorrhizal (do not receive large benefits from AMF) (Busby et al. 2011). This may be a product of possessing long fibrous short lived roots that are less dependent on AMF for survival (Owen et al. 2013). The facultative nature of invasive grasses may lead to decreased AMF diversity (Busby et al. 2013; Martínez-García et al. 2011). This combined with the annual life cycle of *Bromus* species may lead to associations with an AMF community consisting of relatively rapid colonizers that produce mainly intraradicle hyphae (family Glomeraceae) (Allen et al. 2003; Maherali and Klironomos 2007). Whereas woody species may also make associations with AMF species that colonize more slowly but produce a larger amount of extra-radicle foraging hyphae that are associated with increased nutrient acquisition (family Gigasporaceae; Allen et al. 2003; Maherali and Klironomos 2007; Hart and Reader 2002).

Adenostoma fasciculatum, a dominant chaparral shrub, is unique in that it can form associations with both AMF and ectomycorrhizae (EM, Allen et al. 1999). EM dominated soils are often associated with mesic communities whereas AMF are predominant in arid and semi-arid systems (Allen et al. 1995). It is likely that EM

associations with *A. fasciculatum* occur more readily in relatively wet periods when the soil is moist. If invasive grasses are rapidly depleting soil moisture, they may be indirectly decreasing EM colonization. As a result of greater mycorrhizal dependence, it is likely that *A. fasciculatum* selects a different AMF community from invasive grasses (Busby et al. 2013). Therefore, interspersion of invasive annuals may decrease colonization of host-specific EM and AMF by depleting soil moisture and increasing inoculum pressure of AMF species associated with invasive grasses. However, the ability to 'switch' from AMF to EM associations may make *A. fasciculatum* more flexible in forming mycorrhizal associations than other chaparral shrubs. The possibility of unique mycorrhizal associations (both AMF and EM) may make *A. fasciculatum* a good candidate for restoration efforts. Type conversion could induce drastic shifts in AMF diversity and community composition, creating barriers to restoration and re-establishment.

The overarching goal of this dissertation is to understand water use, root and mycorrhizal dynamics of invaded chaparral communities that may inform restoration efforts. More specifically, I aim to address the following four objectives: **(1)** examine how differences in root development relate to soil-water dynamics between a chaparral shrub and an invasive grass; **(2)** determine how invasion affects fungal community composition; **(3)** explore how invasive-conditioned soils affect the growth of chaparral shrub seedlings and associated fungal communities; **(4)** detect if invasive grass water-use strategies are detrimental to shrub seedling success.

In my first chapter, I used *in situ* soil volumetric water content and manual minirhizotron imagery to track root development and soil water status of a native chaparral (*Adenostoma fasciculatum*) and an invasive grass (*Ehrharta calycina*). I also used sap flow sensors to measure transpiration of *A. fasciculatum* and normalized difference vegetation index (NDVI – a measure of canopy greenness) to measure aboveground activity of *Ehrharta calycina*. I found that the invasive grass depleted soil moisture earlier in the season than the native shrub yet there were not differences in the peak of aboveground activity. Additionally, the invasive grass produced longer roots at shallower depths in soil profile than the native shrub. In my second chapter, I examined 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 (AMF). I used high-throughput sequencing to uncover fungal communities of AMF, non-AMF symbionts, pathogens and saprotrophs inhabiting roots and soils associated with invasive grasses and native shrubs. I found that native shrubs hosted a richer and more abundant community of symbiotic fungi (both AMF and other fungi) compared to invasive grasses. For my third chapter, I developed a greenhouse experiment to test how invasive and native conditioned soils affected the growth response of a native chaparral shrub, *Adenostoma fasciculatum*. Inoculation with native soil 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. Additionally, seedlings grown with invasive and native inoculum did not have different growth responses, but both produced more biomass than a sterile control. In my

fourth chapter, I planted *A. fasciculatum* seedlings in the field and maintained three levels of invasive grass cover to assess how the presence of invasive grasses affects soil water availability and the survival of native seedlings. I found that higher invasive grass cover was associated with higher rates of seedling mortality and lower biomass production. Additionally, in the full invasive removal plots, I observed higher levels of soil moisture at 35 cm, which may potentially help shrub seedlings persist through the summer drought. Overall, this research suggests that competition between invasives and natives is more important for invasive persistence than shifts in fungal communities while also illustrating that joining tools and perspectives from the diverse fields of molecular microbial ecology and plant physiological ecology can provide a more comprehensive understanding about how systems respond to change.

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Chapter 1

Differences in root phenology and water depletion by an invasive grass explains persistence in a Mediterranean ecosystem

Abstract

Flexible phenological responses of invasive plant species under a changing climate may increase their ability to establish and persist. A key aspect of plant phenology is the timing of root production and how it coincides with canopy development as well as subsequent water-use. The timing of these events within species and across communities could influence the invasion process. I examined above- and below-ground phenology of two widespread species in the southern California, the native shrub, *Adenostoma fasciculatum,* and the invasive perennial grass, *Ehrharta calycina* to investigate relative differences in phenology and water use. I used normalized difference vegetation index (NDVI) to track whole-canopy activity across the landscape and sap flux sensors on individual chaparral shrubs to assess differences in aboveground phenology of the invasive grass and a chaparral shrub. To determine differences in belowground activity, I used soil moisture sensors, minirhizotron imagery, and stable isotopes. The invasive grass, *Ehrharta calycina*, depleted soil moisture earlier in the spring and produced longer roots at multiple depths early in the growing season than the native shrub, *A. fasciculatum*. However, *A. fasciculatum* produced longer roots in the top 10 cm of soil profile in May. Aboveground activity of the two species peaked at the same time. The fact that *E. calycina* had longer roots earlier in the season suggests that invasive plants may gain a competitive edge over natives through early activity, while

also depleting soil moisture earlier in the season. Depletion of soil moisture earlier by *E. calycina* suggests that invasive grasses could accelerate the onset of the summer drought in chaparral systems, assuring their persistence following invasion.

Introduction

Shifts in vegetation composition due to invasion are an aspect of global change that alters ecosystem processes and function across the world (D'Antonio and Vitousek 1992; Walther et al. 2002; Gonzalez et al. 2010). Terrestrial plant invasion often leads to vegetation community type conversion, such as from native shrubland to invasive grassland, which can be accompanied by changes in soil resource availability. Globally, Mediterranean ecosystems may face the greatest losses of biodiversity because of their susceptibility to multiple global change drivers, such as precipitation variability and biotic introductions (IPCC 2013; Sala et al. 2000).

Invasive grasses are establishing and persisting post-disturbance in the dominant Mediterranean-type shrubland in California, the chaparral (Stylinski and Allen 1999; Keeley and Brennan 2012; Dickens and Allen 2014; Meng et al. 2014). Invasive grasses often possess flexible resource acquisition strategies that can facilitate rapid phenological responses, which may enable them to invade chaparral systems (Ashbacher and Cleland 2015, Willis et al. 2010; Wolkovich and Cleland 2014). Specifically, these strategies could include acclimation to earlier spring temperatures, unseasonably early rains, and the ability to respond to an increase in nutrient availability (Willis et al. 2010). Flexible responses to precipitation could make invasive plants stronger competitors in a changing climate compared to native shrubs, especially when climate interacts with global change

drivers that promote invasion, such as frequent fire, vegetation removal, or anthropogenic nitrogen deposition (Willis et al. 2010; Bradley et al. 2010; D'Antonio and Vitousek 1992; Fenn et al. 2010). Loss of chaparral vegetation to invasive grasses could affect ecosystem structure both above- and below-ground, with potential cascading effects on ecosystem services (Ehrenfeld 2010). To improve my ability to predict risk to invasion and vegetation type conversion in California's chaparral, it is critical to address gaps in understanding related to how phenology enables invasion success in the chaparral and the relationship between above- and below-ground phenology in invaded systems.

In Mediterranean ecosystems, the frequency and magnitude of rain events has the potential to affect the production of fine roots for some vegetation types (Palacio and Montserrat-Martí 2007). Root phenology might enable invasion success through differences in the timing of root development with respect to resource availability (McCormack et al. 2014). Specifically, invasive grasses, whether annual or perennial, may escape drought through the production of short-lived, dense, fine roots for rapid water and nutrient uptake (Williamson et al. 2004a, b; Wolkovich and Cleland 2011; Wainwright et al. 2012). Alternatively, drought tolerant shrubs may be highly dependent on seasonal precipitation events for recharge through the soil profile (Schwinning and Ehleringer 2001), and are likely to possess long-lived, relatively less efficient fine roots (Chen and Brassard 2013). In a high elevation Mediterranean forest, root growth preceded aboveground activity as soil moisture and temperature were increasing (Kitajima et al. 2010). Further, if rain events are not large enough for deep recharge, shrubs may engage in hydraulic redistribution (the movement of water from wetter to

drier regions of soil) from wet upper layers to deep drier layers to maintain existing plant physiological function and foliage throughout the summer drought (Querejeta et al. 2003, 2007, 2009; Ryel et al. 2004; Kitajima et al. 2013). These phenology patterns and rooting architecture may allow chaparral shrubs to better tolerate Mediterranean-climate summer drought.

Although multiple studies have shown that invasive plants may display flexible phenological responses, most studies focus on aboveground responses (Willis et al. 2010; Wainwright et al. 2012). When belowground work is included, the inherent challenge of studying root activity can limit understanding of belowground dynamics (Smith et al. 2014; Wilson 2014, Palacio and Montserrat-Martí 2007; Steinaker and Wilson 2008; Steinaker et al. 2009; Du and Fang 2014; McCormack et al. 2014, 2015). Generally, grass roots of these Mediterranean-type ecosystems tend to be shallow, and plants senesce early in the growing season (e.g., Davis and Mooney 1985; Eliason and Allen 1997; Hooper and Vitousek 1998) whereas shrubs including *Adenostoma fasciculatum* sustain leaves during the dry season, depending on deep roots that penetrate cracks in the bedrock (e.g., Hubbert et al. 2001; Egerton-Warburton et al. 2003). To my knowledge, no studies to date integrate invasion ecology with simultaneous measurements of above- and below-ground phenology. I emphasized temporal dynamics at a fine scale to understand water use through the profile and over time (Allen et al. 2007), to determine if belowground phenological activity differs from aboveground landscape-scale phenology using remote sensing and stand-level phenology using sap-flux measurements. These species characterize the differences in rooting depth and aboveground phenology shifts of

other grass-invaded, type-converted shrublands, where grasses senesce early in the growing season compared to shrubs (Dickens and Allen 2014; Rundel 2018; Williamson et al. 2004b; Davis and Mooney 1985).

In this study, I examined normalized difference vegetation index (NDVI), a measure of greenness, across the landscape coupled with sap-flux measurements to assess phenological differences between the most abundant invasive grass (*Ehrharta calycina*) and the dominant native chaparral shrub (*A. fasciculatum*) at my study site. Most native chaparral shrubs, including my study species are evergreen, meaning that they maintain a relatively constant NDVI throughout the year, whereas invasive grasses senesce in summer causing them to exhibit larger seasonal variations in NDVI (Gamon et al. 1995). Therefore, my unique approach allows us to disentangle the phenological differences between *A. fasciculatum* and *E. calycina*, by using *in situ* transpiration (sap-flux) measures and NDVI, respectively. To determine if there was a rooting phenology offset, I contrasted aboveground phenology with intensive root image and *in situ* environmental and physiological measurements at one site to differentiate *A. fasciculatum* and *E. calycina* water relations and root and shoot phenology. I also explored what water source (surface or deep) *A. fasciculatum* was accessing using stable isotopes. I predicted that (1) *E. calycina* will deplete soil moisture at shallower depths given (2) production of shallower and longer roots as compared to *A. fasciculatum*. I also predicted that (3) *A. fasciculatum* will be able to access deeper water sources at the onset of the summer drought, potentially driving (4) later peak aboveground production in *A. fasciculatum* as compared to *E. calycina*.

Methods

Site Description

The study was conducted in the San Gabriel Mountains at San Dimas Experimental Forest (34 12' N, 117 46' W, 50 km east of Los Angeles) at 830 meters A.S.L. The soils consist of loam in the A horizon (0-8 cm), gravely sandy loam in the C (8-43 cm), and weathered bedrock in the Cr (43-53) with a parent material of residuum weathered from granodiorite (Web Soil Survey, 2016). The soils possess many rock outcroppings and have moderate concentrations of macronutrients (total N = 0.17%, Ulery et al. 1995; extractable P = 30 ug/g and extractable K = 200 ug/g, Egerton-Warburton et al. 2001). The site exhibits a typical Mediterranean climate with cool winters, variable winter rainfall, and hot, dry summers. Mean annual precipitation is 68 cm, however during my seven-month study period (November 2015 – June 2016) which occurred over one growing season there was a total of 41 cm of precipitation. Mean annual temperature is 14.4 º C and summer temperatures regularly exceed 37.8º C but minimum winter temperatures rarely drop below – 3º C (Dunn et al. 1988). The site consists primarily of chaparral shrubland, which is one of the most widespread vegetation types in California (Parker et al. 2016), but some areas were deliberately type converted by seeding *E. calycina* to grassland during the 1960s (Dunn et al. 1988). Overall the site is composed of chaparral species from the genera, *Salvia, Arctostaphylos, Eriogonum, Rhamnus* and *Ceanothus. E. calycina* is by far the most abundant invasive grass at this site, though there are also species of *Bromus* and *Avena*. Adjacent nearly monotypic stands of native shrub, *A. fasciculatum,* or the invasive perennial grass, *E. calycina,* were

chosen for investigation. *A. fasciculatum* is a tall (> 2m), long-lived (>60 yr) shrub, while *E. calycina* is relatively short-statured (< 75 cm) and short-lived (~5 yrs). I manually removed all *E. calycina* that was present in the *A. fasciculatum* stand before the start of the experiment (about 15 individuals, taking care to minimize soil surface disturbance) and continued to remove subsequent seedlings for the duration of the experiment. *A. fasciculatum* is widespread and dominant throughout California chaparral, and *E. calycina* is an abundant invasive grass primarily on the coast (e.g., Cushman et al., 2011).

Environmental measurements

I deployed and maintained volumetric water content (VWC) sensors (CS-616, Campbell Scientific Inc., Logan, UT) from December 2015 until June 2016 in one stand of invasive and one stand of native vegetation. The stands were adjacent and on the same soil type, slope, and aspect. I chose a site that was relatively level (<10% slope) to facilitate instrument installation. The two stands were 10 m apart and each plot within the stand was at least 5 m from other plots to avoid edge effects. Because of the intensive nature of root observations coupled with plant and soil observations, only the two stands were studied.

Three replicate soil moisture were installed 30 cm deep either underneath the root crown individual *A. fasciculatum* shrubs, under monotypic *E. calycina* or under bare soil where I manually removed the grass vegetation. I co-located soil moisture sensors with minirhizotron tubes. Additionally, for *A. fasciculatum,* I chose three individuals that were ~ 5 m away from each other to avoid overlapping root systems. Bare soil and *E. calycina* plots were 1 m^2 and paired and adjacent with an unsampled edge of 0.5 m around bare

plots to avoid edge effects. I compensated for changes in albedo and surface temperature resulting from grass removal by the replacing grass leaf litter on ground. Within the grass and bare sub-plots, soil VWC sensors (CS-650, Campbell Scientific Inc., Logan, UT) were deployed at 30 cm depth. For each plot type (n = 3), I calculated diurnal soil VWC and applied a two-week running average to remove spikes caused by rain events. Daily precipitation data for the entirety of the study period was acquired from PRISM (PRISM Climate Group, Oregon State University, http://prism.oregonstate.edu, created 3 July 2017).

Aboveground phenological measurements

To understand the phenological activity at the landscape scale, I used remotely sensed imagery sourced from the Operational Land Imager (OLI) onboard Landsat 8 (Roy et al. 2014). I acquired level 2 image top of atmosphere reflectance data using Google Earth Engine (GEE (Chander et al. 2009; Schmidt et al. 2013; Dong et al. 2016; Gorelick et al. 2017). I extracted normalized difference vegetation index (NDVI) values for a 30 m pixel (with ca. 70% grass cover), in which my site was located from all available Landsat 8 images with less than twenty percent cloud cover between October 10th, 2015 until June 20th, 2016 (n = 16). Seasonal differences in NDVI that I observe can be primarily attributed to *E. calycina* since the surrounding shrub vegetation is evergreen and therefore maintains relatively consistent NDVI throughout the seasons (Gamon et al. 1995; Park et al. 2018). I compared NDVI measures of *E. calycina* with transpiration measures of *A. fasciculatum.* I fit a harmonic regression to the NDVI values using the 'harmonic.regression' function in the 'HarmonicRegression' package in R to account for

erroneous NDVI values and increase accuracy of my ability to detect peak NDVI values (Lueck et al. 2015).

To measure transpiration, stem sap flux velocity was measured from January to June 2016 using custom built 10-mm Granier-style thermal dissipation probes (Granier 1987) singly or in pairs on *Adenostoma fasciculatum* individuals (n = 7). Outputs were recorded every 30 s and averaged every 5 min using a datalogger (Campbell CR-10x, Campbell Scientific Inc., Logan, UT). Probes were inserted at the widest knot-free point of the stem 10-35 cm above ground and insulated with a reflective mylar wrap as well as silicone caulking. Conducting sapwood area was determined in December 2017 by taking stem cross-sections, staining active xylem with a dilute solution of safranin, and examining sections at 50X magnification (Sano et al. 2005). Non-conducting sapwood area was determined to be negligible and stem sap flow was calculated by scaling flux velocity by stem cross-sectional area at the point of probe insertion. Stand transpiration was calculated by normalizing stem sap flow by stem basal area across instrumented shrubs. I applied a 14-day running average to transpiration data to capture overall trends. I normalized both my 14-day running average stand transpiration and harmonic regression NDVI values to the maximum of each value to compare changes in response to the peak of both *A. fasciculatum* (stand transpiration) and *E.calycina* (NDVI).

Belowground imagery (root length)

Seasonality of root length was followed using a manual minirhizotron (MMR, http://www.rhizosystems.com/Home.php). Sequential below-ground images were captured using wireless 100X digital camera that runs through a transparent 5-cm

diameter tube buried in the soil (MMR, Rhizosystems, LLC). Three MMR tubes were installed under *A. fasciculatum* and three under *E. calycina* at a 45° angle to the soil surface, capturing root standing crop from 0-40 cm below ground. I installed tubes in August 2015 to allow the soil to settle around the tubes and fine roots to grow for prior to data collection. Each tube had an airtight seal to prevent water from accumulating and had an additional PVC covering to prevent light from entering the tube. Imagery was taken bi-weekly at consecutive windows from December 2015 until May 2016. I recorded eighty 6.75 mm x 9.00 mm images for each tube at every time step that were then organized into a mosaic using Rootview (Rhizosystems, LLC) for a total of 6,240 images. An example of a raw image can be found in Figure 1.1. Image processing was done using Rootfly (Version 2.0.2, https://cecas.clemson.edu/~stb/rootfly/, Wells and Birchfield, Clemson University, SC, USA), where I measured lengths and diameters of all roots observed. I aggregated both the root length into monthly observations and bin by true depth from surface level.

Stable isotope analysis

Depending on the time of year and the type of plant, stem water reflects the water source a plant is using spatially and temporally (Ehleringer et. al. 1991). To determine if *A. fasciculatum* is accessing water sources at different depths seasonally, I collected rainwater and well water in February 2017 as well as stem samples from *A. fasciculatum* in February 2017 and June 2017. Plant stems were collected directly from live plants and immediately placed in 10 mL vacutainers (BD, Franklin Lakes, NJ), which were capped and sealed with parafilm to prevent evaporation. Samples were subsequently frozen at -

20°C until analyses. Water was extracted from plant stem samples using a cryogenic vacuum distillation line for at least 60 min for stems (Ehleringer et al. 2000; West et al. 2006). Stable isotopic composition of oxygen ($\delta^{18}O$) analyses were conducted at the FIRMS at the University of California, Riverside using a TC/EA (Thermo Scientific) interfaced with an isotope ratio mass spectrometer (Delta V Advantage, Thermo Scientific). Values for $\delta^{18}O$ are reported in delta notation (‰) relative to the Vienna Standard Mean Ocean Water (V-SMOW) standard:

$$\delta = \left/ \frac{R_{sample}}{R_{standard}} - 1 \right. \times 1000 \quad (1)$$

Statistical analyses

Repeated-measures ANOVA were fit to diurnal soil VWC and root length data using the 'lmer' and 'anova' functions from the 'lme4' and 'stats' R packages (Bates et al., 2015, R Core Team 2017, Supplemental Table S1). To structure the repeated-measures ANOVAs and account for temporal autocorrelation, I built linear mixed effects models and included measurement number (day) as a random effect. Then, I used the 'anova' function on the linear mixed effects model object. For diurnal VWC, candidate predictor variables were vegetation type, month, and vegetation type: month. Replicates were treated as random effects to account for spatial variation. To examine the interaction of vegetation type and month for the VWC model, I calculated the estimated marginal means (least-squares means) using the 'emmeans' function with Tukey's adjustment from the 'emmeans' package in R (Lenth, 2019). For the root length model (0-40 cm),

candidate predictor variables were vegetation type, month, vegetation type: month, depth, and depth: vegetation type. I used month as a predictor variable because it averages the VWC or root length across multiple measurements which removes some of the temporal autocorrelation structure of the data. Again, I included measurement number (measurements were taken bi-weekly) and replicate as random effects. For model selection, I used the 'step' function from the 'lmerTest' package on full models to do a backwards elimination of fixed effects using AIC. I retained full models for both VWC and root length, as Δ AIC values were less than two.

I used four repeated measures ANOVAs for each depth bin (0-10 cm, 10-20 cm, 20-30 cm, and 30-40 cm) with root length as the response variable and vegetation type and month as the predictor variables. Measurement number (time) and replicate were treated as random effects. I calculated estimated marginal means with Tukey's adjustment to compare the interaction between vegetation type and month on root length for each depth.

ANOVAs were fit to the isotope data using the 'anova' function from the stats' R package (R Core Team 2017). Tukey's pairwise comparisons were performed on $\delta^{18}O$ isotope data using the 'TukeyHSD' function in the 'stats' package (Supplemental Table 1.5). All data conformed to expectations of normality of residuals and homoscedasticity of variance. For $\delta^{18}O$ analyses, source (well water, rain water, stem water in February, and stem water in June) was the predictor variable and $\delta^{18}O$ values were the response variable. Analyses were conducted using R version 3.2.1 (R Core Team, 2017). All data

and analyses used to generate these results are publicly available as a redistributable R package: https://github.com/bmcnellis/SDEF.analysis.

Results

Environmental variables

Soil moisture (measured as VWC) in the *A. fasciculatum* stand (native vegetation) at 30 cm depth began to increase after rain events (< 5 cm) in mid-December 2015 (Figure 1.2A). Equipment failure prevented assessment of soil moisture for *E. calycina* (invasive vegetation) and bare soil plots until mid-January when it was observed that soil moisture across the site steadily increased with multiple rain events (Figure 1.2A). I did not include measurements in my statistical analyses before mid-January when sensors in all plots were operating. Soil moisture peaked under native and invasive vegetation during the middle of March, and the peak was marginally less under invasive vegetation.

Based on repeated measures ANOVA, soil moisture values were significantly different by month and there was a significant interaction between vegetation type and month ($p = 0.0002$ and 0.0007, respectively, Supplemental Table 1.1). VWC was not significantly different between vegetation type alone over all months ($p = 0.8844$, Supplemental Table 1.1), but native vegetation had significantly higher soil moisture values than plots with bare ground for January, February and March ($p = 0.0009$, 0.0039 and 0.0023, respectively, Figure 1.2A, Supplemental Table 1.2). Soil moisture under invasive vegetation was higher than under plots with bare ground in January, February and March ($p = 0.0035$, 0.0004 and 0.0007, respectively, Figure 1.2A, Supplemental

Table 1.2). In April, there was no difference in soil moisture under invasive vegetation and bare ground ($p = 0.2814$, Figure 1.2A, Supplemental Table 1.2). Soil moisture did not differ under native and invasive vegetation until April ($p = 0.0191$, Figure 1.2A, Supplemental Table 1.2). After April, soil moisture remained higher under native than invasive vegetation in May and June ($p = 0.0001$ and 0.0002, respectively, Figure 1.2A, Supplemental Table 1.2). In May, soil moisture in plots with bare ground was higher than under invasive vegetation ($p = 0.036$, Figure 1.2A, Supplemental Table 1.2).

Aboveground phenology –

A. fasciculatum responded to rain events (> 5 cm) in early January, with concomitant increased soil water availability and transpiration as measured by sap flux sensors (Figs. 2a and 2b). Landscape-level aboveground activity (NDVI), which is primarily driven by grass activity, reached its' peak on March 27th and the aboveground activity of *A. fasciculatum* (transpiration using sap-flux) peaked around the same time on March 30th (Figure 1.2A).

Belowground imagery (root length) –

During the study period we observed a total of 233 roots of *A. fasciculatum* with a mean root length of 4.05 mm in the viewing area (0-40 cm in the soil profile around the 5-cm diameter tube). Whereas for *E. calycina* there was a total of 1,596 roots with a mean root length of 4.11 mm. Repeated measures ANOVA showed that root length was affected by the interaction between vegetation type and month ($p = 0.00001$; Figure 1.3; Supplemental Table 1.3), but not by vegetation type alone ($p = 0.2935$; Figure 1.3; Supplemental Table 1.3). Additionally, the interaction of vegetation type with depth

affected root length ($p = 0.0045$; Figure 1.3; Supplemental Table 1.3). Specifically, Invasive grasses produced longer roots than native shrubs across our entire observation area within the soil profile (0-40 cm) in December 2015 ($p = 0.0090$; Figure 1.3; Supplemental Table 1.4). I did not observe significant differences in root length across all depths (0-40 cm) during any months after December 2015 ($p > 0.05$; Figure 1.3; Supplemental Table 1.4).

The interaction of vegetation type with month affected root length at 0-10 cm ($p = 0.0001$; Figure 1.3; Supplemental Table 1.3). Invasive vegetation produced longer roots than native vegetation at 0-10 cm and 20-30 cm in December 2015 ($p = 0.002$ and 0.0097, respectively; Figure 1.3; Supplemental Table 1.4). There were no differences in root length at 0-10 cm in January, February or March 2016 ($p = 0.3501$, 0.9545 and 0.5774; Figure 1.3; Supplemental Table 1.4), however native shrubs possessed longer roots at 0-10 cm in May 2016 ($p = 0.0037$; Figure 1.3; Supplemental Table 1.4). Invasive vegetation produced longer roots at 10-20 cm than native vegetation in February 2016 ($p = 0.0501$; Figure 1.3; Supplemental Table 1.4). We didn't observe any significant differences in root length between vegetation types at 20-30 and 30-40 cm for any months ($p > 0.05$; Figure 1.3; Supplemental Table 1.4).

Stable isotope analysis –

I used the $\delta^{18}O$ signatures from the two water sources, rainwater and well water (i.e. groundwater), coupled with $\delta^{18}O$ signatures from *A. fasciculatum* stem water to discern what sources of water *A. fasciculatum* was accessing during the wet and dry

seasons. Well and rain water samples had similar signatures that were not significantly different ($P > 0.05$, Supplemental Table 1.5). Stems collected from *A. fasciculatum* in February coinciding with abundant precipitation had significantly lower $\delta^{18}O$ than well-water samples, but not lower than rainwater samples ($p < 0.05$, Figure 1.4, Supplemental Table 1.5). In contrast, the stems collected from the same individuals in June, coinciding with the onset of the summer drought, had significantly higher $\delta^{18}O$ than winter rainy season stem samples and rain water samples ($p < 0.001$, Figure 1.4, Supplemental Table 1.5).

Discussion

Intensive measurements over time showed that invasive vegetation depleted soil moisture more rapidly toward the end of the rainy season than both native vegetation and bare ground. Greater depletion of soil moisture under *E. calycina* starting in April and continued into the summer drought when compared to *A. fasciculatum* supports my first hypothesis. I expected that aboveground activity (NDVI) at the site level – representing invasive grass activity (Gamon et al., 1995) – would peak before *A. fasciculatum* aboveground activity (transpiration), but instead found that they peaked around the same time. I also found support for our prediction that *E. calycina* would produce longer roots at shallower depths than *A. fasciculatum*, because *E.calycina* possessed longer roots in December 2015. Lastly, I expected that *A. fasciculatum* would access deep water sources at the onset of the summer drought, meaning that the $\delta^{18}O$ signature of the stems collected in June would match the well water. However, there was little support for this hypothesis as the $\delta^{18}O$ signature from the stems in June were distinct from both water

sources. Overall, I found differences in root length at shallow depths and depletion of soil moisture suggesting that these plant species can differentially affect soil water balance.

During the rainy season (January – March), there were no differences in soil moisture between native and invasive vegetation types, suggesting that *E. calycina* was not using water more rapidly than *A. fasciculatum* at 30 cm. Both invasive and native vegetation types had higher soil moisture than bare ground, which indicates that the presence of any vegetation decreases runoff and increases soil water infiltration. This dynamic shifted later in the growing season because soil moisture under invasive vegetation dropped below soil moisture under native vegetation and bare ground starting in April, coinciding with the end of the rainy season. This could lead to an acceleration of the onset of the summer drought in areas where invasive grasses are present (Davis and Mooney, 1985; Eliason and Allen, 1997; Williamson et al., 2004a; 2004b).

Since there were no differences in soil moisture between native and invasive vegetation during the rainy season, this naturally lends to similar peak activity times in aboveground activities. This could be driven by the fact that both species are perennial. Soil moisture increased in response to rain events in early January, and *A. fasciculatum* responded with increases in root length and increases in aboveground activity. This indicates that *A. fasciculatum* activity is driven by precipitation and more specifically that root responses precede or occur simultaneously with aboveground transpiration responses, as was also observed in high elevation Mediterranean forest (Kitajima et al., 2010). The invasive grass had higher root length values in December than in January,

before any substantial rain events (> 2 cm), suggesting that it was able to take advantage of small increases in soil moisture and that root activity precedes aboveground activity.

There was support for my hypothesis that invasive grasses would deplete soil moisture more rapidly and produce roots earlier at shallow depths than *A. fasciculatum*, allowing them to gain a competitive edge through early phenological activity or seasonal priority effects (Wainwright et al., 2012; Willis et al., 2010). *E. calycina* produced longer roots at multiple depths earlier in the growing season than *A. fasciculatum*. The early presence of longer roots of *E. calycina* suggests that this species may respond rapidly to rain events, but *A. fasciculatum* response was delayed. Our observation that *E. calycina* possessed longer roots earlier in the growing season shows some support for the idea that this invasive plant may be able to respond to early rains faster than natives (Willis et al., 2010).

The invasion literature suggests that functional differences between two species would make them less likely to compete for resources (Funk et al., 2008), and I expected that *A. fasciculatum's* deep rooting strategy would allow it to access deep water whereas *E. calycina* would access shallow soil moisture. However, there was an overlap of root depth between *A. fasciculatum* and *E. calycina* in monospecific stands. *A. fasciculatum* root length increased and were longer than *E. calycina's* roots at the onset of the summer drought at shallow depths in the soil profile (0-10 cm), indicating that *A. fasciculatum* is extracting remaining moisture from the last rain events. Under conditions where *A. fasciculatum* has an exotic grass understory, the grass might have an overlapping resource depletion zone with native shrubs resulting in direct competition for water

(Chakraborty and Li, 2009). Yet since this study was conducted in monospecific stands, further observations of potential root overlap in mixed stands are needed.

During the dry season, the $\delta^{18}O$ signature of the stem water indicates that *A. fasciculatum* is taking up enriched water. There are a few potential explanations for this, one being that *A. fasciculatum* is accessing a third source of water that I did not sample. However, if *A. fasciculatum* is primarily using remaining surface water in June, the surface soil water may be heavier in ^{18}O due to evaporative enrichment after precipitation ceases. The enriched $\delta^{18}O$ signature could also suggest that *A. fasciculatum* is using a mix of water from deep and surface sources because the roots are still active at both depths. Higher April soil moisture values for *A. fasciculatum* plots than *E. calycina* but not than bare ground plots, suggest that something other than hydraulic redistribution is driving differences in soil moisture between native and invasive vegetation. One possibility is that *A. fasciculatum* transpires less than *E. calycina*, which is a pattern that has been seen in other comparisons of invasive and native water-use (Cavaleri and Sack, 2010; Williamson et al., 2004b). Also, differences in root length, especially at shallow depths, between the vegetation types could be driving differences in soil moisture. It is also important to note that previous studies corroborate that *A. fasciculatum* produces roots much deeper (> 1m deep) than my observation zone (Williamson et al., 2004a; Schenk and Jackson, 2002; Kummerow et al., 1983). As the technology to study roots develops, future research in the chaparral should make efforts to monitor roots below 40 cm.

Conclusions

Using a combination of intensive measurements of individual plants during the growing season and NDVI to assess phenology at the landscape scale, I measured differences in soil moisture associated with vegetation type, which could be driven by differences in rooting strategies. While my intensive studies did not allow me to measure additional plant species, they are supported by other observations of moisture depletion by invasive grasses (Davis and Mooney 1985; Eliason and Allen 1997; Williamson et al., 2004a; 2004b). The depletion of soil moisture earlier in the season by *E. calycina* provides support for my hypothesis that *E. calycina* can deplete soil moisture rapidly and I also found support for my hypothesis that *E. calycina* would produce more, longer roots at shallower depths earlier in the growing season than *A. fasciculatum* (Frazer and Davis 1985). Subsequently, these invasive grasses have the potential to accelerate the onset of the summer drought and decrease deep soil water recharge, which could inhibit the re-establishment of native shrubs and further increase vulnerability to invasion. Potentially, native shrubs may redistribute water between deep and shallow depths sustaining continued root activity (Querejeta et al., 2003, 2007, 2009; Kitajima et al., 2013), however I did not find evidence that *A. fasciculatum* is accessing deeper water sources at the onset of the summer drought compared to the rainy season. These results suggest that in a mixed stand, native chaparral shrubs and invasive grasses would have overlapping resource depletion zones (Chakraborty and Li, 2009). Competition for water is one mechanism that has been cited as a cause of persistence of invasive grasses in desert shrublands (DeFalco et al., 2007). Although I did not measure direct competition for

water in this study, the overlapping depletion zone indicates that an invasive understory would directly compete with a native overstory; this may explain why native shrubs have not been able to recolonize and the invasive grass stand has been stable for over six decades.

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Figures

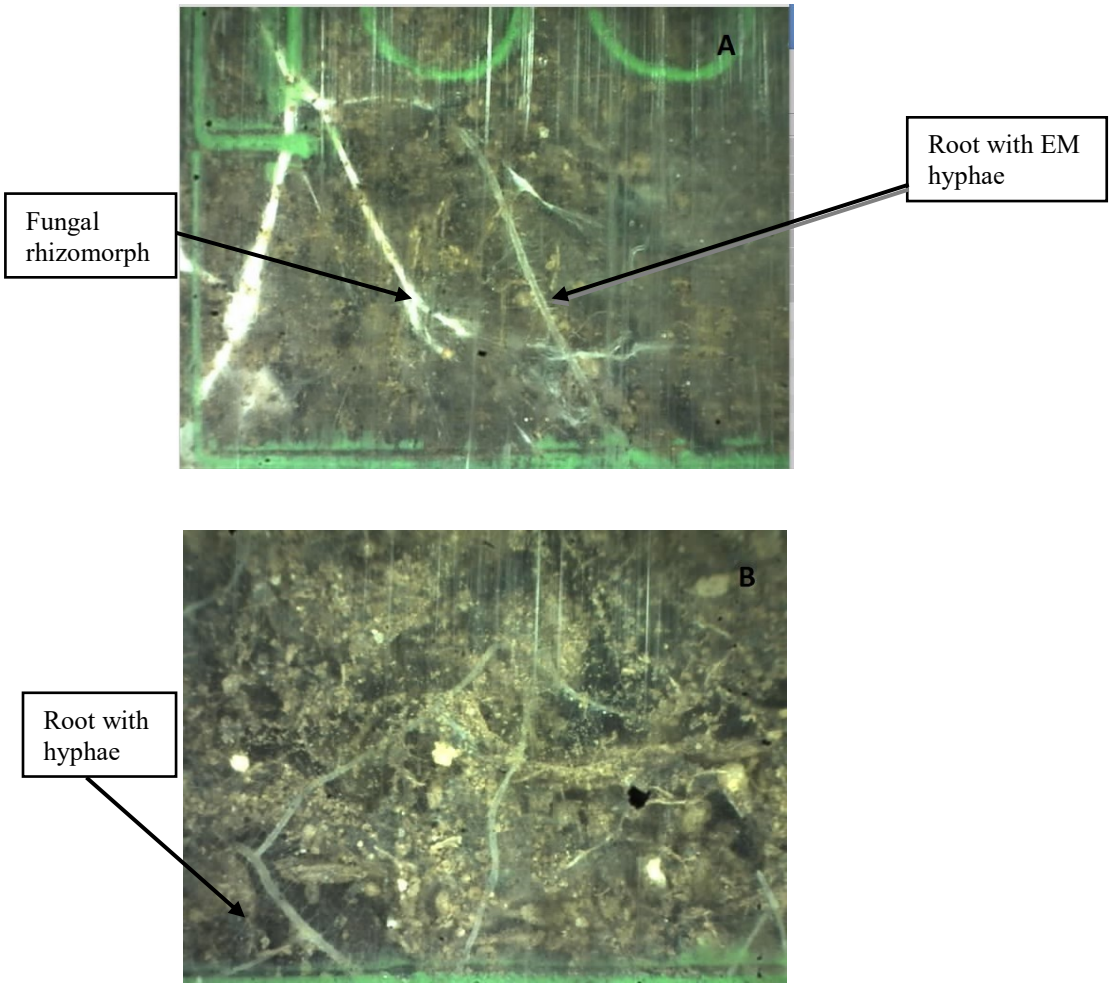

Figure 1.1: Example of image from manual minirhizotron (50x magnification) displaying (A) *A. fasciculatum* roots and EM (ectomycorrhizal) hyphae in February 2016 at ~35 cm depth and (B) *E. calycina* roots and arbuscular mycorrhizal hyphae in December 2015 at ~25 cm depth.

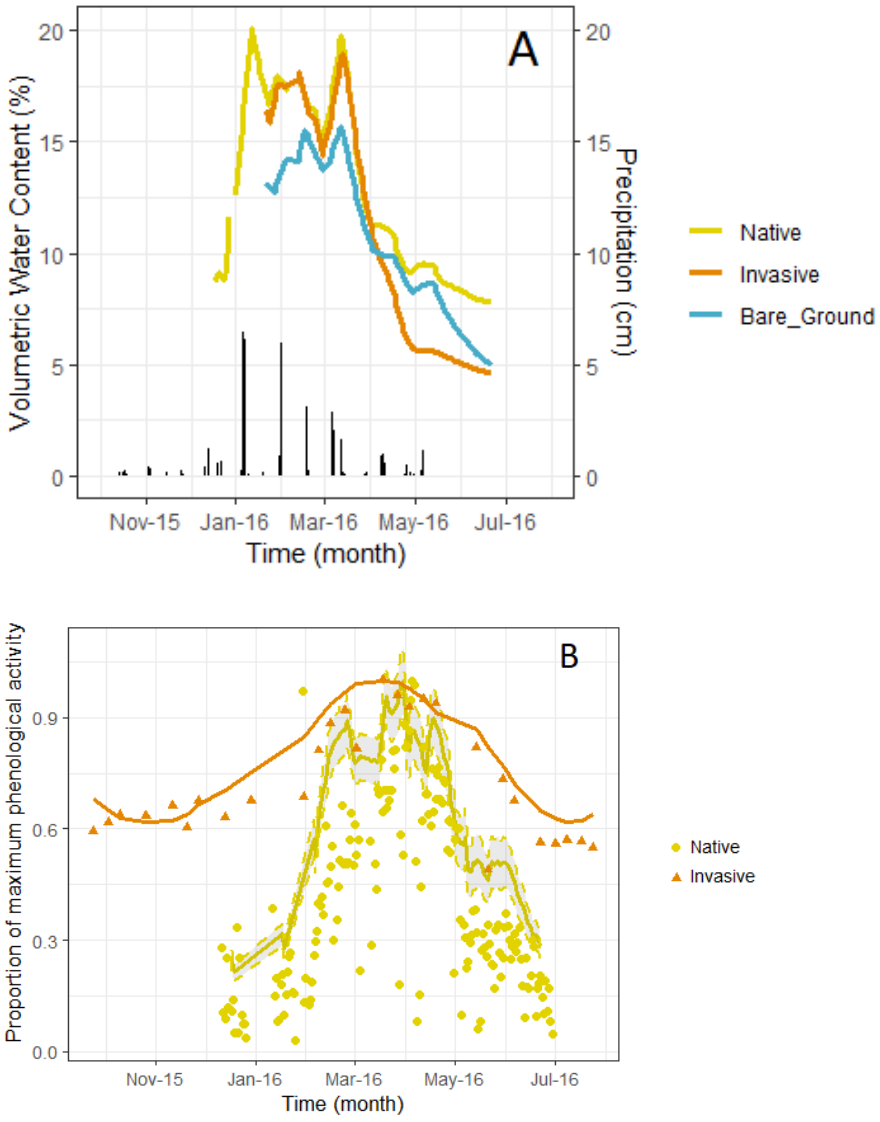

Figure 1.2: (A) Two week running average of volumetric water content at 30 cm depth for native (*A. fasciculatum*), invasive (*E.calycina*), and bare ground (all vegetation removed) plots (n = 3). Bars represent precipitation events derived from PRISM data. (B) Two-week running average of aboveground activity measured as transpiration of native vegetation in grey (*A. fasciculatum)* and aboveground activity measured as NDVI fit to a harmonic regression for the study site (representing invasive grass activity) normalized as a percentage of the maximum observed value for each. Points are values used to fit harmonic regression (NDVI - orange triangles) or two-week running average (transpiration - yellow circles). Standard error for transpiration values (n = 7) is displayed around two-week running average (solid yellow line) as a grey ribbon with dashed yellow-lines.

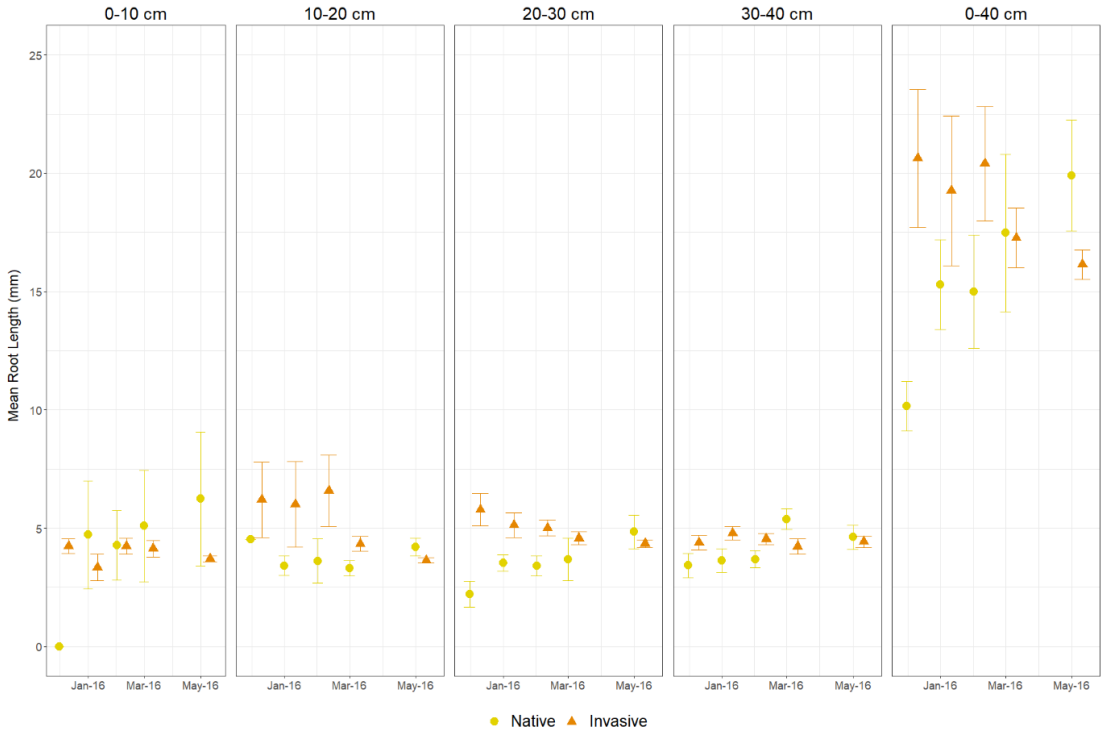

Figure 1.3: Monthly mean root length (mm) of native vegetation (*A. fasciculatum*; n = 3) and invasive vegetation (*E. calycina*; n = 3) at four depths within the soil profile and the total observation area (0-40cm in the soil profile) from the beginning of the rainy season to the beginning of the dry season. Significance at $p < 0.05$ based on estimated marginal means is denoted by *. Significance at $p < 0.05$ for vegetation type is based on repeated measures ANOVA is denoted by ++.

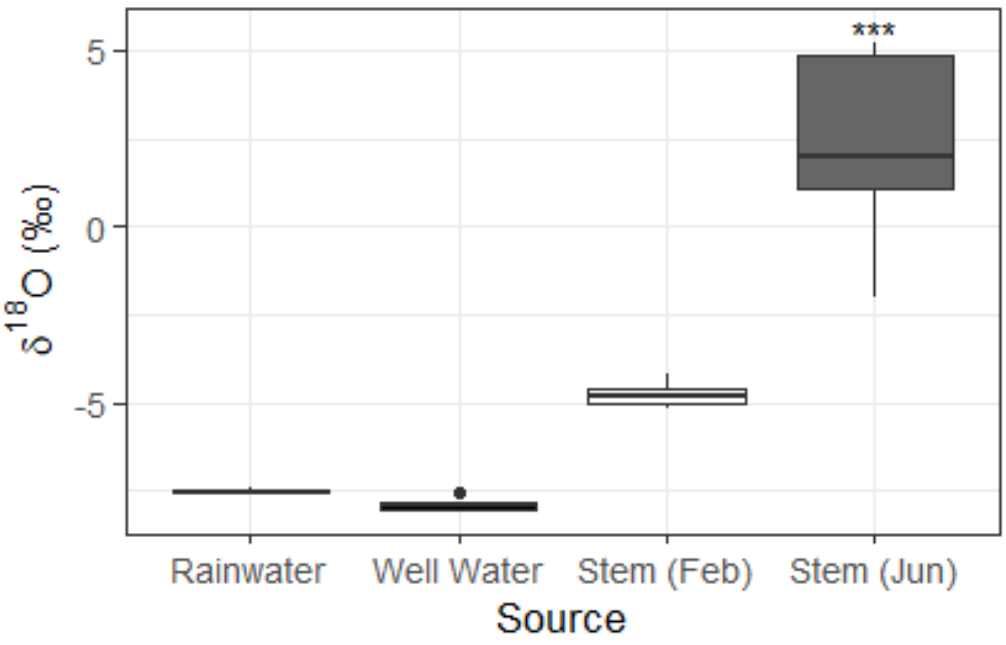

Figure 1.4: Oxygen isotope composition ($\delta^{18}O$) from four water sources (n = 6), rainwater collected in February, well water to represent groundwater collected in February, *A. fasciculatum* stems collected during the rainy (February) season and dry (June) season. Dots are outliers. Significance at $p < 0.005$ is denoted by ***.

Supplemental Tables

Supplemental Table 1.1: P-values and F-values (in parenthesis) for Repeated Measures ANOVAs. Significant values ($P < 0.05$) in bold.

Response Variable	**Vegetation Type**	**Month**	**Vegetation Type X Month**
Soil VWC	0.8844 (0.13)	**0.0002 (169)**	**0.0007 (67)**

Supplemental Table 1.2: Outputs from estimated marginal means (least-squares means), testing for an effect of vegetation types at the different month levels, with soil VWC the response variable.

Contrast	Month	p-value
Native - Bare ground	Jan-16	**0.0009**
Native - Invasive	Jan-16	0.9221
Bare ground - Invasive	Jan-16	**0.0035**
Native - Bare ground	Feb-16	**0.0039**
Native - Invasive	Feb-16	0.8178
Bare ground - Invasive	Feb-16	**0.0004**
Native - Bare ground	Mar-16	**0.0023**
Native - Invasive	Mar-16	0.9477
Bare ground - Invasive	Mar-16	**0.0007**
Native - Bare ground	Apr-16	0.4649
Native - Invasive	Apr-16	**0.0191**
Bare ground - Invasive	Apr-16	0.2814

Native - Bare ground	May-16	0.2653
Native - Invasive	May-16	**0.00001**
Bare ground - Invasive	May-16	**0.0041**
Native - Bare ground	Jun-16	**0.0036**
Native - Invasive	Jun-16	**0.0002**
Bare ground - Invasive	Jun-16	0.7026

Supplemental Table 1.3: P-values and F-values (in parenthesis) for Repeated Measures ANOVAs with root length as the response variable. Significant values ($P < 0.05$) in bold.

Depth	**Vegetation Type**	**Depth**	**Month**	**Vegetation Type X Month**	**Vegetation Type X Depth**
0-40 cm	0.2935 (1.43)	0.2998 (1.22)	**0.0086 (3.41)**	**0.00001 (8.17)**	**0.0045 (4.34)**
0-10 cm	0.9712 (0.0015)	NA	**0.0031 (4.06)**	**0.0001 (5.88)**	NA
10-20 cm	**0.0313 (4.66)**	NA	0.3604 (1.09)	**0.0446 (2.46)**	NA
20-30 cm	0.0953 (4.39)	NA	0.7049 (0.54)	0.0782 (2.16)	NA
30-40 cm	0.8589 (3.32)	NA	0.1045 (1.95)	**0.0378 (2.59)**	NA

Supplemental Table 1.4: Outputs from estimated marginal means (least-squares means), testing for an effect of vegetation types at depth levels for the different months, with root length as the response variable.

Contrast	Month	Depth	p-value
Native - Invasive	Dec-15	0-40 cm	**0.0090**
Native - Invasive	Jan-16	0-40 cm	0.1479
Native - Invasive	Feb-16	0-40 cm	0.0726
Native - Invasive	Mar-16	0-40 cm	0.6387
Native - Invasive	May-16	0-40 cm	0.0992
Native - Invasive	Dec-15	0-10 cm	**0.0020**
Native - Invasive	Jan-16	0-10 cm	0.3501
Native - Invasive	Feb-16	0-10 cm	0.9545
Native - Invasive	Mar-16	0-10 cm	0.5774
Native - Invasive	May-16	0-10 cm	**0.0037**
Native - Invasive	Dec-15	10-20 cm	0.4983
Native - Invasive	Jan-16	10-20 cm	0.0573

Native - Invasive	Feb-16	10-20 cm	**0.0501**
Native - Invasive	Mar-16	10-20 cm	0.5059
Native - Invasive	May-16	10-20 cm	0.2450
Native - Invasive	Dec-15	20-30 cm	**0.0097**
Native - Invasive	Jan-16	20-30 cm	0.1646
Native - Invasive	Feb-16	20-30 cm	0.0799
Native - Invasive	Mar-16	20-30 cm	0.3847
Native - Invasive	May-16	20-30 cm	0.8470
Native - Invasive	Dec-15	30-40 cm	0.8620
Native - Invasive	Jan-16	30-40 cm	0.3271
Native - Invasive	Feb-16	30-40 cm	0.2146
Native - Invasive	Mar-16	30-40 cm	0.1702
Native - Invasive	May-16	30-40 cm	0.8495

Supplemental Table 1.5: P-values for Tukey's pairwise comparison for $\delta^{18}O$ sources. Significant values ($P < 0.05$) in bold.

$\delta^{18}O$ Source	Stem (Feb)	Stem (June)	Rain (Feb)	Well
Stem (Feb)				
Stem (June)	**< 0.001**			
Rain (Feb)	0.084	**< 0.001**		
Well	**< 0.001**	**0.012**	0.983	

Chapter 2
Fungal community assembly in soils and roots under plant invasion and nitrogen deposition

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. I 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. I found a higher richness and abundance of rhizophilic (e.g. Glomeraceae) and edaphophilic (e.g. Gigasporaceae) AMF with increasing soil NO_3. My 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.

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; Egerton-Warburton, Johnson and Allen 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; Egerton-Warburton, Johnson and Allen 2007). Additionally, some invasive plants exhibit relatively low AMF dependence which could decrease the presence of AMF (Busby et al. 2013, 2011; Hawkes et al. 2006). 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 intra-radical 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, Johnson and Allen 2007). At P-limited sites, fertilization often increases AMF productivity and diversity (Treseder and Allen 2002; Egerton-Warburton, Johnson and Allen 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. (2018, 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; Varela-Cervero et al. 2016a; Varela-Cervero et al. 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).

In this study, I 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). I 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. I 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.

Methods

Site Description

I 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. I 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 I 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).

Host plants

In March 2016, I 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). I sampled the 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, I 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 I sampled from adjacent stands (>5 meters but <10 meters 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).

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 2.2.

I extracted DNA from soils (~0.25g/sample) and roots (~0.15g/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 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.

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). I 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. I 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 fit 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).

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. (2011). 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. 2017). 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 seconds at 95

°C, 30 seconds at 55 °C, 4 minutes 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 I used 100 nM of each indexing primer, only one reaction was conducted per sample and only 15 total cycles were performed. I 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). I 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 2x300 mode.

Bioinformatics

I 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, I joined paired-end of raw reads with ea-utils (Aronesty 2011). I then checked joined read quality across read length with FastQC (Andrews S. 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, I used the forward raw read and checked quality with FastQC (SSU 201 bp; Andrews S. 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). I 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). I 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 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. I further filtered my OTU tables (0.005% across the table) recommended in Bokulich et al. 2013 to remove rare (presumed spurious) OTUs. For both loci, I 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 http://dx.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 .

Functional group assignment

To examine responses of the general fungal community (ITS2), I 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, I 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 I 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. I 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. I retained 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. I manually BLASTed the 'no blast hits' against the NCBI database to ensure that these OTUs were not Glomeromycotina. Therefore, I did not retain the 96 OTUs (35%) with 'no blast hit' in any of my downstream analyses. To interpret responses of the AMF community (SSU) I assigned families of Glomeromycotina to AMF functional groups: rhizophilic, edaphophilic and ancestral using AMF resource allocation patterns defined in previous studies (Table 2.1). Families that did not fall into rhizophilic or edaphophilic groups were placed in the ancestral group (Table 2.1). I did not include sequences reportedly identified as *Geosiphon pyriformis*, of which there were only two observations, in any of the functional groups.

Beta Diversity

For each locus, I 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. I 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, I 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, I could include all variables as the homogeneity of groups dispersion was met for every predictor variable.

Generalized linear models

I used generalized linear models (GLMs) to test my hypotheses about fungal functional group responses to invasion and elevated soil N concentrations. I built GLMs using the 'glm' function in the MASS package in R (Venables and Ripley 2002). I fit 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. I used the 'stepAIC' function from the MASS package to further select these models for parsimony (Venables and Ripley 2002). I used separate models for roots and soils by functional group richness and relative abundance of each locus, resulting in twenty-four models.

Joint taxa distribution modeling

To understand how environmental variables structure AMF relative taxonomic abundance, I 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.

I 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. I performed 200,000 Marcov 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 1,000 posterior samples. I 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. I considered environmental predictors as fixed effects and individual sample as a random effect. I checked for model convergence by visually assessing the MCMC trace plots. I used the posterior distributions of each predictor and calculated the probability that it was different from zero. I considered parameters "significant" when their posterior probabilities had at least a 90% probability of being different from zero ($p = 0.1$). I 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 my models, while also understanding how much variation in family abundance can be explained by each of my environmental variables as well as random processes.

Results

Percent colonization

Roots of invasive annual grasses had higher colonization by AM and non-AM hyphae than native shrub roots (72% $\pm$ 4 (mean $\pm$ SD)) invasive and 5% $\pm$ 33 native, $P = 0.003$, and 56% $\pm$ 38 and 8% $\pm$ 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 I did observe more vesicles in invasive roots than in native roots (11% and 2%, respectively; P = 0.002). I did not observe EMF colonization in *A. fasciculatum* roots.

SSU sequences (AMF)

I observed a total of 277 OTUs, 181 of which were assigned taxonomy after performing BLAST against the MaarjAM database. For sequences with assigned taxonomies, I 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. I observed the following 9 genera: *Glomus, Acaulospora, Archaeospora, Paraglomus, Scutellospora, Claroideoglomus, Geosiphon, Ambispora,* and *Redeckera*. Of those genera, only 2 OTU's were identified as *Geosiphon pyriformis* which I removed from subsequent analyses, because it did not fall into any AMF functional grouping. Family relative read abundances can be found in Supplemental Table 2.2. I 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).

ITS2 sequences (general fungal community)

I 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 I had removed AMF to avoid overlap between my 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, Teloschitaceae, Lobariaceae, Lecideaceae – contain lichenized fungal species.

Beta Diversity

AMF beta diversity differed by site ($R^2 = 0.04$, $P = 0.02$, Figure 2.1). 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 ($R^2 = 0.04$, $P = 0.01$, Figure 2.2) and the interaction between host plant and sample type ($R^2 = 0.03$, $P = 0.04$, Figure 2.2).

Functional group responses

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; Figure 2.3A; Supplemental Table 2.1). Rhizophilic AMF richness and relative abundance in roots was

negatively correlated with soil NH_4 concentrations (P = 0.003 and 0.016, R^2 = 0.81 and 0.82, respectively; Supplemental Table 2.1). 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; Supplemental Table 2.1). 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; Figure 2.3A).

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, Figure 2.3A, Supplemental Table 2.1), while richness did not differ between these plant roots (P = 0.26, R^2 = 0.60, Figure 2.3A). 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, Supplemental Table 2.1). Richness of edaphophilic AMF in roots was positively correlated with soil NO_3 (P = 0.04, R^2 = 0.60, Supplemental Table 2.1). 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; Supplemental Table 2.1).

Ancestral AMF

Native roots had greater relative read abundance, but not richness of ancestral AMF families when compared to invasive (P = 0.006 and 0.2, R^2 = 0.76 and 0.66,

respectively; Supplemental Table 2.1). 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, $R^2 = 0.66$, Supplemental Table 2.1). 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; Supplemental Table 2.1).

Non-AMF Symbionts

Non-AMF symbionts – including EMF – had greater richness (Figure 2.4A) and relative abundance in native roots ($P = 0.002$ and 0.003, $R^2 = 0.95$ and 0.98, respectively; Supplemental Table 2.1). Non-AMF symbiont richness, but not abundance, was also greater in native soils (Figure 2.4B, $P = 0.035$ and 013, $R^2 = 0.95$ and 0.98, respectively; Supplemental Table 2.1). Non-AMF symbiont richness in roots was negatively associated with soil NH_4 and NO_3 concentrations ($P = 0.001$ and 0.001, respectively, $R^2 = 0.95$; Supplemental Table 2.1). 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, $R^2 = 0.98$; Supplemental Table 2.1).

Pathogens

Pathogen fungi were relatively more abundant in invasive grass roots (Figure 2.4A, $P = 0.011$, $R^2 = 0.58$; Supplemental Table 2.1), however richness did not differ (Figure 2.4B, $P = 0.63$, $R^2 = 0.60$). Pathogen richness (Figure 2.4B) and relative abundance were greater in invasive soils ($P = 0.001$ and 0.001, $R^2 = 0.84$ and 0.82,

respectively; Supplemental Table 2.1). 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; Supplemental Table 2.1). 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; Supplemental Table 2.1).

Saprotrophs

Saprotroph relative abundance was greater in invasive soils (P = 0.001, R^2 = 0.73), however saprotroph richness was greater in native soils (P = 0.001, R^2 = 0.65, Figure 2.4B; Supplemental Table 2.1). 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; Supplemental Table 2.1). Saprotroph richness in soils negatively correlated with soil NO_3 concentration (P = 0.022, R^2 = 0.65; Supplemental Table 2.1). Root saprotroph richness was higher in native roots when compared to invasive (P = 0.03, R^2 = 0.54; Supplemental Table 2.1).

Taxonomic abundance responses

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 ($P \leq 0.1$, Supplemental Table 2.4), however I found no significant differences between sites in soils (Supplemental Table 2.5). Relative read abundance for all AMF families in roots was

positively correlated with soil NO_3 concentrations ($P \leq 0.1$, Supplemental Table 2.4). I observed increases in relative abundance of Acaulosporaceae, Archaeosporaceae, Claroideoglomeraceae, Diversisporaceae, Glomeraceae, and Paraglomeraceae in roots with increasing soil P concentrations ($P \leq 0.1$, Supplemental Table 2.4). 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 ($P \leq 0.1$, Supplemental Table 2.5). Relative abundance of Acaulosporaceae, Ambisporaceae, and Claroideoglomeraceae in soils increased with increasing soil NH_4 concentrations ($P \leq 0.1$, Supplemental Table 2.5).

Variance partitioning

Environmental predictors (host plant, site, NH_4, NO_3, pH, and P) explained 92% $\pm$ 7% of the variance in the AMF root community model (Figure 2.5A, Supplemental Table 2.6). 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 (Supplemental Table 2.6, Figure 2.5A). Soil NO_3 concentrations explained the largest amount of model variance in the root model (33% $\pm$ 4%, Figure 2.5A, Supplemental Table 2.6). In soil communities, total environmental predictors explained 92% $\pm$ 7% of model variance (Figure 2.5B, Supplemental Table 2.7). Soil P concentrations explained the largest amount of the variance ranging from 35% $\pm$ 14% of the variation in the soil model (Figure 2.5B, Supplemental Table 2.7).

Discussion

Overall my findings suggest that while the same pool of AMF mutualists is available for both *A. fasciculatum* and the invasive grasses I 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 my 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 my microscopic observations of higher AMF colonization in invasive roots than native roots. I expected that invasive grasses would host more rhizophilic AMF taxa, however these taxa were relatively more abundant and richer in native shrub roots. I hypothesized that invasive grasses would harbor fewer pathogens but did not find strong support for this. Instead, I found that pathogenic fungi were relatively more abundant in invasive roots 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 *Adenostoma fasciculatum*. I expected that invasive hosts would interact with soil N, resulting in decreased richness and abundance of edaphophilic AMF, but I have little support for this hypothesis. While my beta-diversity analyses suggest that habitat filtering alters AMF abundances between soils and roots, I observed an even greater separation between the rest of the fungal community between native and invasive plant roots.

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). I 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 I 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, I 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 (Hart *et al*. 2016; Hijri et al.

2006; Öpik et al. 2008). This suggests that when these invasive grasses persist, I may see shifts in the relative abundance of taxa, but not a complete turnover of AMF taxa that are present. However I 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 I 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). I sampled during a drought year which likely decreased the presence of AMF and EMF in these soils.

I did not observe effects of site or host plant on any AMF families in roots or soils, but in my functional guild analyses I 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 my 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. I 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). I must note that the family level results from my joint distribution model need to be interpreted cautiously, because I use relative read abundances in these models. The read abundance data I used 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).

My 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).

Pathogenic and other non-AMF fungi

I 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, I 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), I lost potentially valuable data.

However, using only conservative functional group assignments with the confidence levels 'highly probable' and 'probable' protected the integrity of my 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). My 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 my observations of greater relative abundance of saprotrophs in invasive associated soils (De Deyn et al. 2008).

I 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 I use in this study are coarse tools which at best approximate fungal ecological functioning. My 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.

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; Busby et al., 2013). Yet, this must be interpreted cautiously because my AMF colonization contradicts this finding because it suggests that AMF are more abundant in invasive roots. I observed differences in relative abundance and richness of functional groups of AMF between native and invasive root and soil communities. However, in my taxonomic analyses I did not find differences in abundance of any AMF family between native and invasive roots or soils. My 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). My results do not support my hypothesis that invasive grasses would host more rhizophilic

taxa, as rhizophilic AMF were richer and relatively more abundant in native shrub roots. However, I 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). My 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.

I did not find strong support for my 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, I 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. My 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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Tables

Table 2.1: Description of AMF Functional Groups adapted from (Weber *et al*., 2017). 1. (Powell *et al.* 2009); 2. (Hart and Reader 2002); 3. (Varela-Cervero *et al.* 2015); 4. (Varela-Cervero *et al.* 2016a); 5. (Varela-Cervero *et al.* 2016b)

Functional Group	**Intraradical Hyphae**	**Extraradical Hyphae**	**Families**
Rhizophilic	High	Low	Glomeraceae[1, 2, 3, 4, 5] Claroideoglomeraceae[1] Paraglomeraceae
Edaphophilic	Low	High	Gigasporaceae[1, 2, 5] Diversisporaceae[1, 5]
Ancestral	Low	Low	Archaeosporaceae Ambisporaceae Acaulosporaceae[1, 2, 5] Pacisporaceae

Table 2.2: 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)

Figures

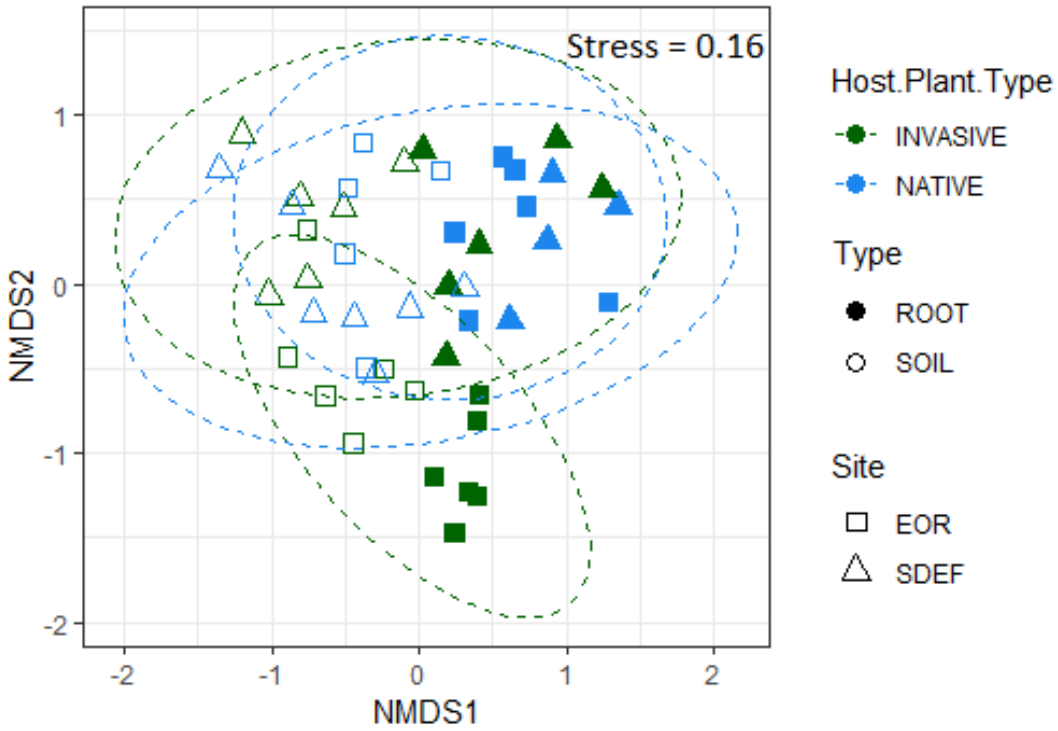

Figure 2.1: AMF (SSU) Bray-Curtis NMDS plot. Color is host plant, shape denotes site: San Dimas Experimental Forest (SDEF) or Emerson Oaks Reserve (EOR) and fill denotes if the community is from a root (solid) or soil (no fill) sample. The stress value is 0.16.

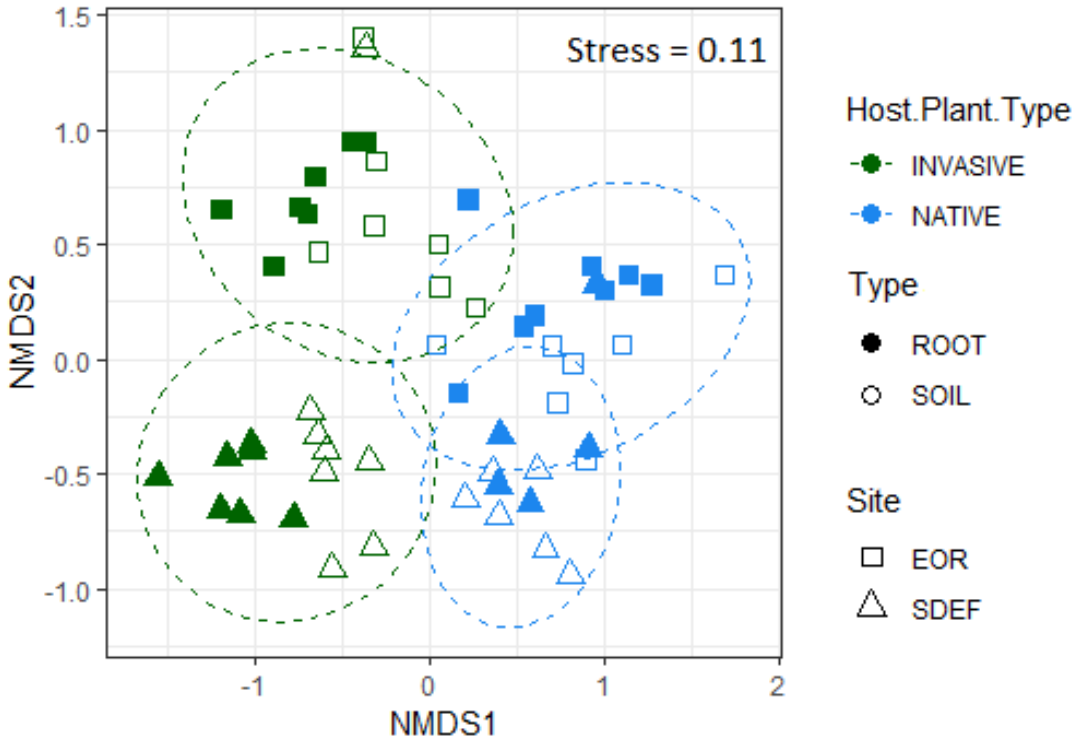

Figure 2.2: General Fungal Community (ITS2) Bray-Curtis NMDS plot. Color is host plant, shape denotes site: San Dimas Experimental Forest (SDEF) or Emerson Oaks Reserve (EOR) and fill denotes if the community is from a root (solid) or soil (no fill) sample. The stress value is 0.11.

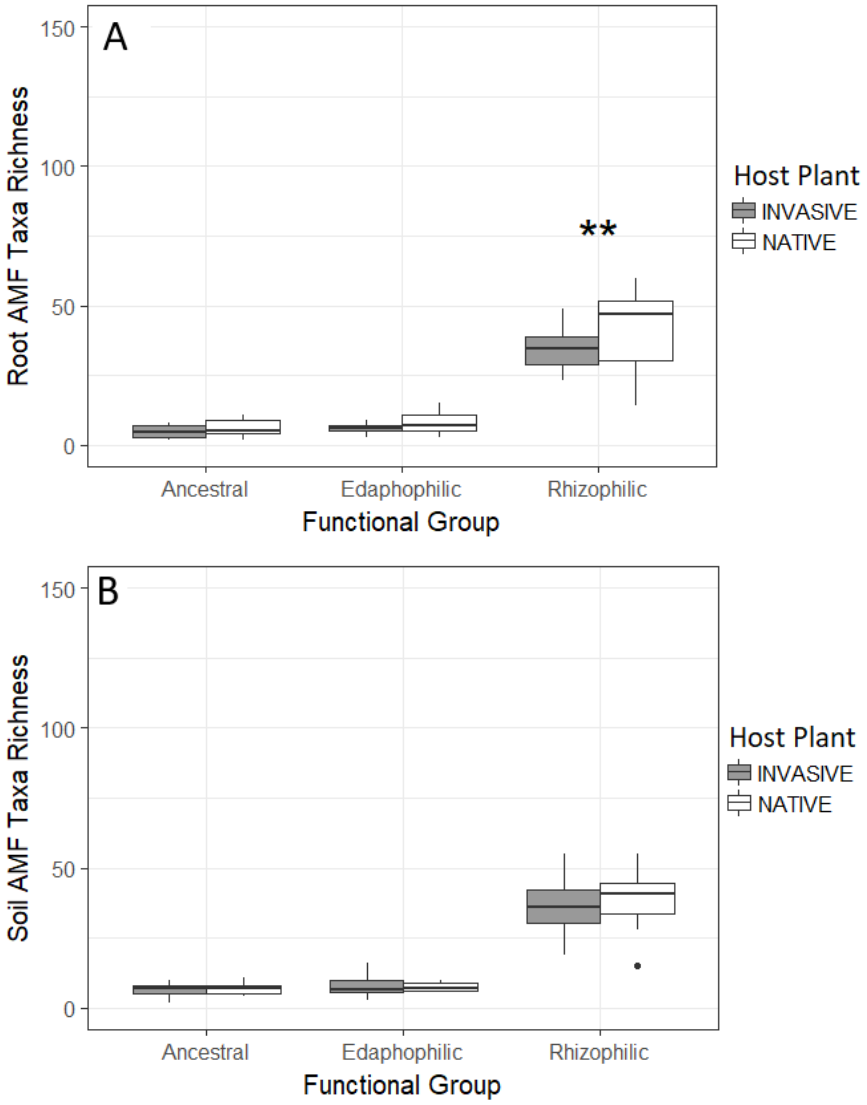

Figure 2.3: SSU or arbuscular mycorrhizal fungal (AMF) root (A) and soil (B) communities by functional group by aggregating species by family using the phylogenetic scheme in Table 2.1. AMF taxa richness is the number of times a unique taxonomic unit is encountered in each sample. *** denotes significant 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 Supplemental Table 2.1.

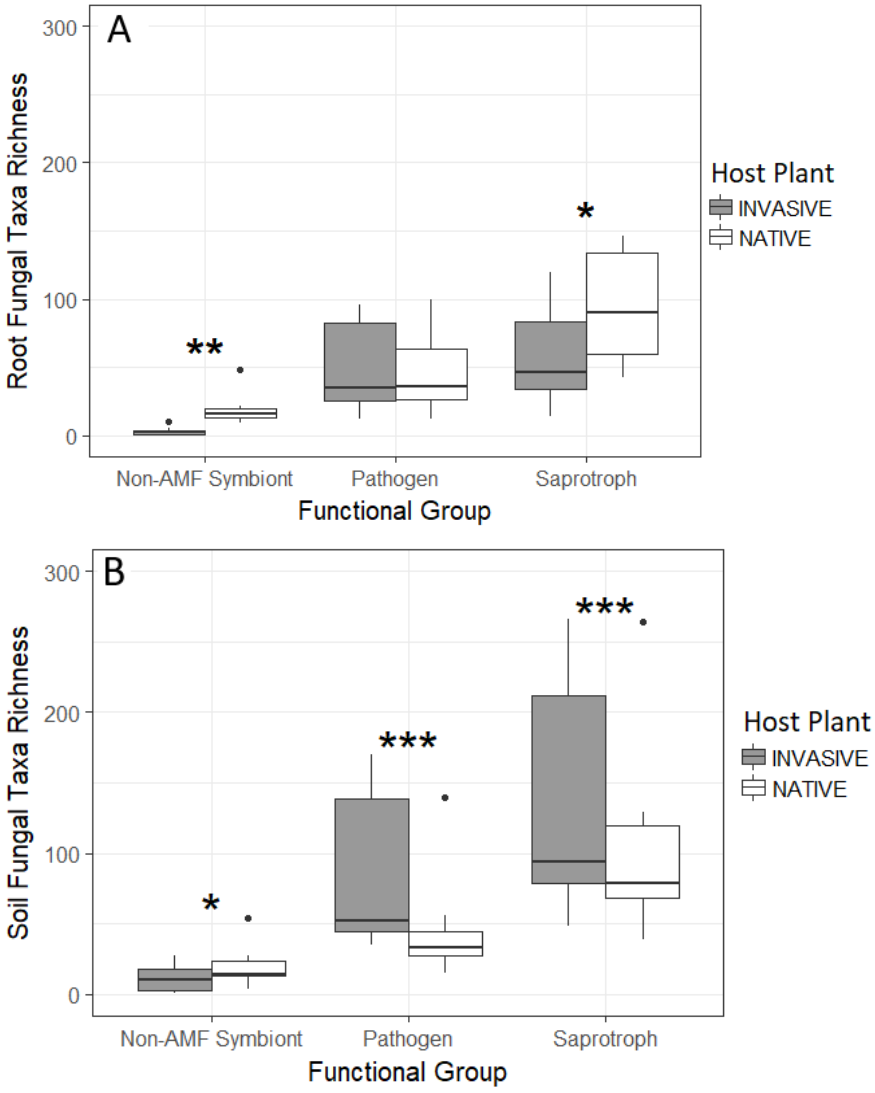

Figure 2.4: ITS or general fungal community root (A) and soil (B) communities by functional group by aggregating species using FUNguild. Fungal taxa richness is the number of times a unique taxonomic unit is encountered in each sample. *** denotes significant difference by host plant at $P < 0.001$, ** denotes significance at $P < 0.01$ and * denotes significance at $P < 0.05$ from GLM outputs in Supplemental Table 2.1.

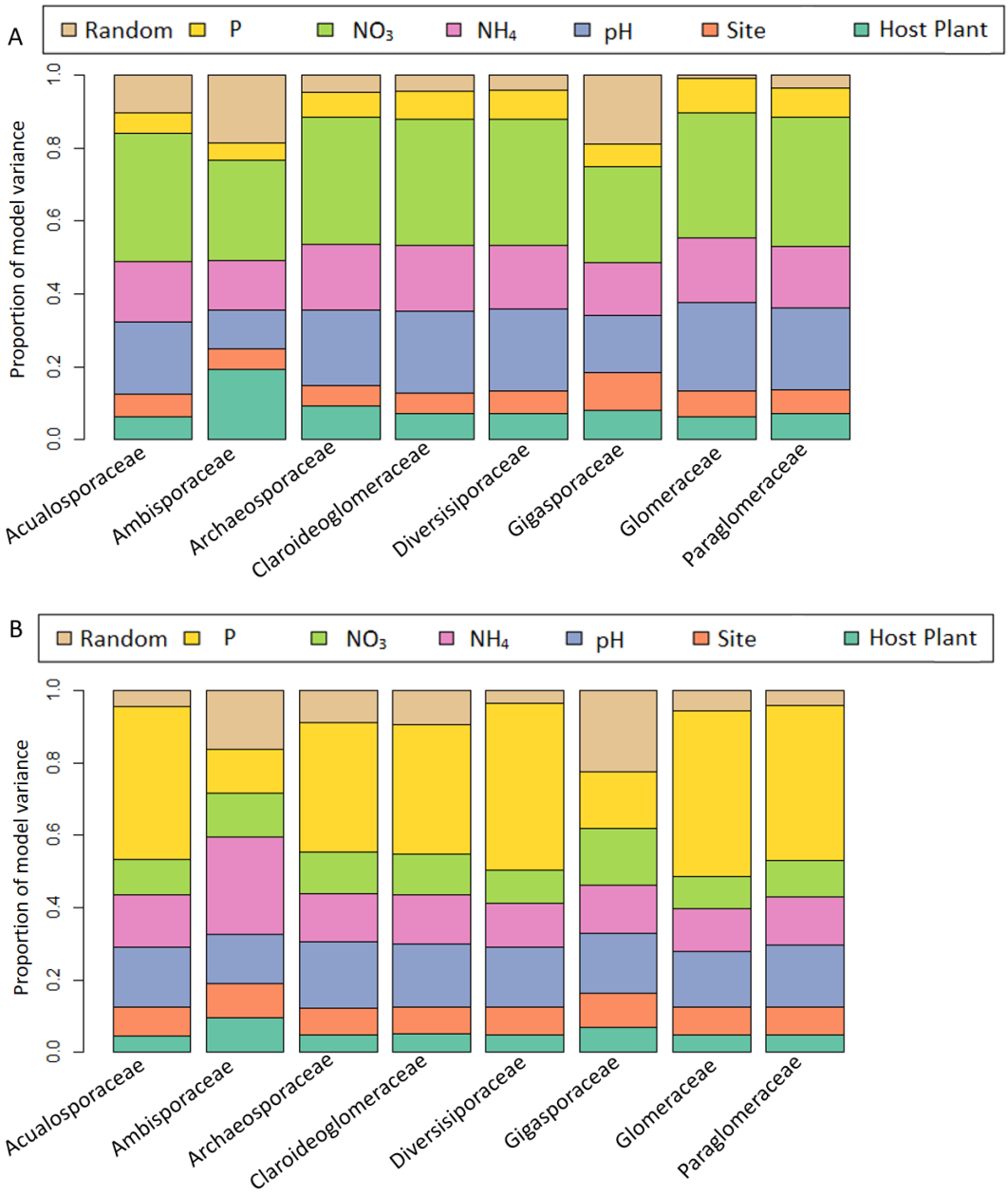

Figure 2.5: Results of variance partitioning for the variation in root (A) and soil (B) AMF relative abundance (at the family level) in response to host plant (native or invasive), site (SDEF or EOR), P, NO_3, NH_4, pH, site, and host plant. Individual sampled was included as the random effect.

Supplemental Tables

Supplemental Table 2.1: Summary of significant outputs from generalized linear models.

Response Variable	Predictor Variable	Direction of Effect	P-value
Root Rhizophilic OTU Richness	Host Plant (Native)	+	0.006
Root Rhizophilic OTU Richness	Site (SDEF)	+	0.035
Root Rhizophilic OTU Richness	NH_4	-	0.003
Root Rhizophilic OTU Richness	NO_3	+	0.001
Root Rhizophilic OTU Richness	Site: NH_4	-	0.010
Root Rhizophilic OTU Richness	Host Plant (Native): NH_4	+	0.001
Root Rhizophilic Relative Read Abundance	Host Plant (Native)	+	0.016
Root Rhizophilic Relative Read Abundance	Site (SDEF)	+	0.034
Root Rhizophilic Relative Read Abundance	NH_4	-	0.005
Root Rhizophilic Relative Read Abundance	NO_3	+	0.002
Root Rhizophilic Relative Read Abundance	Site(SDEF): NH_4	-	0.019
Root Rhizophilic Relative Read Abundance	Host Plant (Native): NH_4	+	0.001
Root Edaphophilic OTU Richness	NO_3	+	0.004
Root Edaphophilic Relative Read Abundance	Host Plant (Native)	+	0.023
Root Edaphophilic Relative Read Abundance	Site (SDEF)	-	0.010
Soil Edaphophilic Relative Read Abundance	NH_4	-	0.033
Soil Edaphophilic Relative Read Abundance	NO_3	+	0.005
Soil Edaphophilic Relative Read Abundance	Host Plant (Native)	+	0.007
Soil Edaphophilic Relative Read Abundance	Site (SDEF)	+	0.019
Soil Edaphophilic Relative Read Abundance	P	+	0.024
Soil Edaphophilic Relative Read Abundance	pH	+	0.011
Soil Edaphophilic Relative Read Abundance	Host Plant (Native): NH_4	+	0.00
Root Ancestral OTU	NH_4	-	0.006

Richness			
Root Ancestral OTU Richness	NO_3	+	0.011
Soil Ancestral OTU Richness	NO_3	-	0.018
Root Ancestral Relative Read Abundance	Host Plant (Native)	+	0.006
Root Ancestral Relative Read Abundance	NO_3	+	0.001
Soil Ancestral Relative Read Abundance	Site (SDEF): NH_4	+	0.020
Root Non-AMF Non-AMF Symbiont OTU Richness	Host Plant (Native)	+	0.002
Root Non-AMF Non-AMF Symbiont Relative Read Abundance	Host Plant (Native)	+	0.003
Soil Non-AMF Non-AMF Symbiont OTU Richness	Host Plant (Native)	+	0.035
Root Non-AMF Non-AMF Symbiont OTU Richness	NH_4	-	0.001
Root Non-AMF Symbiont OTU Richness	NO_3	-	0.001
Root Non-AMF Symbiont Relative Read Abundance	NH_4	+	0.001
Root Non-AMF Symbiont Relative Read Abundance	NO_3	+	0.003
Root Non-AMF Symbiont Relative Read Abundance	Site(SDEF): NH_4	-	0.003
Root Non-AMF Symbiont Relative Read Abundance	Site(SDEF): NO_3	-	0.001
Root Non-AMF Symbiont OTU Richness	Site(SDEF): NH_4	-	0.001
Root Non-AMF Symbiont OTU Richness	Site(SDEF): NO_3	-	0.001
Soil Non-AMF Symbiont OTU Richness	Host Plant (Native)	+	0.035
Soil Non-AMF Symbiont OTU Richness	NH_4	-	0.002
Root Pathogen Relative Read Abundance	Host Plant (Native)	-	0.011
Soil Pathogen Relative Read Abundance	Host Plant (Native)	-	0.001
Soil Pathogen OTU Richness	Host Plant (Native)	-	0.001
Soil Pathogen OTU Richness	Site (SDEF)	+	0.001
Soil Pathogen Relative Read Abundance	Site (SDEF)	+	0.001
Soil Pathogen OTU Richness	Host Plant (Native): Site(SDEF)	-	0.018

Soil Pathogen Relative Read Abundance	Host Plant (Native): Site(SDEF)	-	0.009
Soil Saprotroph Relative Read Abundance	Host Plant (Native)	-	0.001
Soil Saprotroph OTU Richness	Host Plant (Native)	-	0.001
Soil Saprotroph OTU Richness	NH_4	+	0.001
Soil Saprotroph OTU Richness	NO_3	-	0.022
Soil Saprotroph OTU Richness	NH_4	+	0.001
Soil Saprotroph OTU Richness	NO_3	-	0.022
Root Saprotroph OTU Richness	Host Plant (Native)	+	0.029

Supplemental Table 2.2: The relative read abundance for the families detected using SSU loci in invasive and native samples and divided into roots and soil samples. Mean and standard deviation of reads from each family per sample binned by host plant type, across samples are shown (mean ± standard deviation).

Family	Invasive Relative Read Abundance	Native Relative Read Abundance	Invasive Soil Relative Read Abundance	Native Soil Relative Read Abundance	Invasive Root Relative Read Abundance	Native Root Relative Read Abundance
Acaulosporaceae	459 (19±14)	345 (16±8)	302 (25±16)	236 (22±7)	158 (13±9)	110 (10±6)
Ambisporaceae	91 (4±4)	151 (7±5)	62 (5±4)	83 (8±5)	29 (2±4)	68 (6±4)
Archaeosporaceae	335 (14±11)	410 (19±10)	202 (17±13)	177 (16±9)	133 (11±8)	233 (21±11)
Claroideoglomeraceae	495 (21±16)	434 (20±11)	244 (20±20)	175 (16±10)	251 (21±11)	260 (24±11)
Diversisporaceae	889 (37±16)	1091 (50±23)	548 (46±32)	572 (52±22)	341 (28±11)	520 (47±26)
Gigasporaceae	165 (7±5)	143 (7±12)	87 (7±5)	91 (8±15)	78 (7±6)	52 (5±7)
Glomeraceae	5087 (212±102)	4823 (219±72)	2592 (246±133)	2679 (244±78)	2135 (178±40)	2145 (195±58)
Paraglomeraceae	648 (27±15)	643 (29±14)	337 (28±19)	251 (23±7)	312 (26±10)	392 (36±17)

Supplemental Table 2.3: The richness for the families detected using SSU locus in invasive and native samples and divided into roots and soil samples. Mean and standard deviation of OTU richness from each family per sample binned by host plant type, across samples are shown (mean ± standard deviation).

Family	Invasive Richness	Native Richness	Invasive Soil Richness	Native Soil Richness	Invasive Root Richness	Native Root Richness
Acaulosporaceae	70 (19±2)	52 (16±2)	43 (4±2)	36 (3±2)	27 (13±2)	16 (10±1)
Ambisporaceae	16 (4±1)	26 (7±1)	11 (2±1)	15 (1±1)	5 (2±1)	11 (6±1)
Archaeosporaceae	69 (14±2)	77 (19±2)	43 (4±3)	35 (3±2)	26 (11±1)	42 (21±2)
Claroideoglomeraceae	74 (21±2)	68 (20±2)	38 (3±3)	27 (3±1)	36 (21±2)	41 (24±2)
Diversisporaceae	147 (37±4)	161 (50±3)	91 (8±5)	84 (8±2)	56 (28±2)	77 (47±4)
Gigasporaceae	36 (7±1)	26 (7±2)	21 (2±1)	91 (8±2)	15 (7±1)	10 (5±1)
Glomeraceae	776 (212±17)	770 (219±11)	444 (37±22)	410 (37±10)	332 (178±7)	360 (195±12)
Paraglomeraceae	97 (27±2)	96 (29±2)	55 (5±2)	44 (4±1)	42 (26±2)	52 (36±3)

Supplemental Table 2.4: Mean and standard deviation of estimates for the posterior distribution of joint distribution model for AMF in roots at the family level. Parameters estimate the response of fixed effects (host Plant, site, pH, NH_4, NO_3, and P on fungal family relative abundance. Significant parameters are shown in bold (at $p \leq 0.10$) and with a grey background (at $p \leq 0.05$).

	Host Plant		Site		pH		NH4		NO3		P	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Acaulosporaceae	1.82	1.16	1.58	1.52	**3.77**	1.89	3.50	1.73	**5.30**	2.17	**1.80**	1.39
Ambisporaceae	1.39	0.67	0.17	0.85	0.89	0.90	1.16	0.82	**1.84**	0.94	-0.13	0.75
Archaeosporaceae	3.94	1.65	**2.51**	2.32	**6.22**	2.77	6.00	2.74	**8.62**	3.46	**3.26**	2.22
Claroideoglomeraceae	4.29	1.99	**3.26**	2.87	**8.22**	3.52	7.52	3.27	**10.82**	4.32	**4.45**	2.78
Diversisporaceae	7.50	3.56	**6.16**	5.08	**14.44**	6.46	12.88	5.81	**19.00**	7.93	**7.93**	4.87
Gigasporaceae	0.76	0.89	0.91	1.03	**1.36**	1.15	1.32	1.08	**1.97**	1.25	0.52	0.92
Glomeraceae	33.11	17.24	**31.86**	24.06	**70.35**	29.75	61.03	26.49	**88.47**	34.52	**41.02**	23.71
Paraglomeraceae	6.15	2.82	5.13	4.13	**11.81**	5.18	10.43	4.65	**15.61**	6.15	**6.58**	4.02

Supplemental Table 2.5: Mean and standard deviation of estimates for the posterior distribution of joint distribution model for AMF in soils at the family level. Parameters estimate the response of fixed effects (host Plant, site, pH, NH_4, NO_3, and P on fungal family relative abundance. Significant parameters are shown in bold (at $p \leq 0.10$) and with a grey background (at $p \leq 0.05$).

	Host Plant		Site		pH		NH4		NO3		P	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Acaulosporaceae	-0.17	2.94	1.63	3.54	**4.67**	4.32	**4.41**	3.98	-1.51	4.38	-8.70	3.13
Ambisporaceae	-0.43	0.90	-0.12	1.01	0.20	1.30	**1.46**	1.09	-0.18	1.20	-0.04	1.21
Archaeosporaceae	-0.38	1.91	0.89	2.36	**3.31**	2.92	2.79	2.63	-1.41	2.94	-5.26	2.27
Claroideoglomeraceae	-0.29	2.13	0.94	2.51	3.32	3.14	**3.11**	2.89	-1.42	3.26	-5.73	2.64
Diversisporaceae	-0.43	6.07	3.78	7.22	**9.59**	8.78	7.96	8.18	-3.20	8.73	-18.56	6.46
Gigasporaceae	-0.29	0.99	0.50	1.26	1.10	1.49	0.93	1.35	-0.92	1.49	-0.87	1.56
Glomeraceae	-1.75	23.00	15.81	28.95	32.43	36.16	25.10	34.08	-12.10	34.00	-65.52	40.92
Paraglomeraceae	-0.14	3.23	1.98	3.91	**5.35**	4.87	4.59	4.37	-1.71	4.81	-9.67	3.42

Supplemental Table 2.6: Results of variance partitioning analysis. Proportions of total variation explained for roots of each AMF family corresponding to Figure 2.5A.

	Host Plant	Site	pH	NH4	NO3	P	Random
Acaulosporaceae	0.063	0.061	0.199	0.167	0.350	0.059	0.102
Ambisporaceae	0.192	0.058	0.108	0.134	0.275	0.049	0.185
Archaeosporaceae	0.093	0.055	0.207	0.180	0.351	0.066	0.047
Claroideoglomeraceae	0.071	0.058	0.225	0.179	0.346	0.076	0.044
Diversisporaceae	0.071	0.064	0.225	0.173	0.346	0.080	0.042
Gigasporaceae	0.081	0.104	0.157	0.146	0.262	0.061	0.190
Glomeraceae	0.063	0.072	0.241	0.177	0.345	0.093	0.009
Paraglomeraceae	0.071	0.065	0.225	0.171	0.353	0.081	0.035

Supplemental Table 2.7: Results of variance partitioning analysis. Proportions of total variation explained for soils of each AMF family corresponding to Figure 2.5B.

	Host Plant	Site	pH	NH4	NO3	P	Random
Acaulosporaceae	0.046	0.077	0.168	0.143	0.100	0.421	0.045
Ambisporaceae	0.095	0.094	0.136	0.271	0.121	0.121	0.162
Archaeosporaceae	0.048	0.073	0.183	0.134	0.116	0.358	0.089
Claroideoglomeraceae	0.050	0.074	0.174	0.138	0.110	0.359	0.094
Diversisporaceae	0.047	0.077	0.166	0.121	0.093	0.462	0.033
Gigasporaceae	0.069	0.096	0.164	0.134	0.157	0.155	0.226
Glomeraceae	0.047	0.076	0.157	0.117	0.089	0.459	0.055
Paraglomeraceae	0.048	0.078	0.172	0.131	0.101	0.430	0.041

Chapter 3: Native and invasive inoculation sources modify fungal community assembly and biomass production of a chaparral shrub

Abstract

Feedbacks between plants and surrounding soil microbes can contribute to the establishment and persistence of invasive plants as well as limit the success of restoration efforts. In this study, I aim to understand how three sources of soil inocula – native, invasive and sterile – affect the growth response and fungal community composition in the roots of a chaparral shrub, *Adenostoma fasciculatum*. I grew *A. fasciculatum* from seed in a greenhouse with each inoculum source and harvested at six months. I 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 increases the diversity of multiple groups of fungal symbionts present in native seedling's roots 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.

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 (Stylinski and Allen 1999; Keeley and Brennan 2012; Meng et al. 2014; Dickens and Allen 2014). Type 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.

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). Furthermore, invasive 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). However, native perennials are more likely to be dependent on AMF families that associated with increased nutrient acquisition, such as in Gigasporaceae. These families colonize host plants more slowly and produce a larger amount of extraradical foraging hyphae that aid in nutrient acquisition (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 (Hart and Reader 2002; Powell et al. 2009; Varela-Cervero et al. 2015; Varela-Cervero et al. 2016a; Varela-Cervero et al. 2016b; 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 2.1). 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, I 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, I 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. I 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). I 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 host a less diverse community of rhizophilic and edpahophilic AMF 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, I 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.

Methods

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 I sampled in areas where chaparral had recovered, and areas where exotic grasses persisted. I 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. For inoculation, I did not pool replicate samples and instead inoculated each of 15 pots per treatment with each individual replicate separately. I 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 I scarified them in a 10% sulfuric acid solution for ten minutes. For the potting mix, I 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). I steam- sterilized this field soil – sand mixture for 24 hours, held at room temperature for 24 hours, and steam-sterilized for another 24 hours. I placed the soil into sterilized 800 ml Conetainer® pots and mixed with 40g 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 (20g from sterilized invasive and 20g sterilized native collected field soil). I germinated seeds in these Conetainers, thinned to one individual per plot, and harvested at six months. I made efforts to minimize contamination by keeping inoculum treatments separate from one another, while keeping conditions consistent between treatments by rotating pots biweekly.

Percent colonization

At the time of harvest, I reserved 0.15g of fresh roots for DNA extraction and stored them in a -20 °C freezer. I weighed the remainder of the fresh roots for calculating water content of the roots to account for the fresh roots removed for molecular analyses. I placed fresh roots and shoots in coin envelopes, dried them at 60 °C for 48 hours, and weighed them to determine seedling biomass. I rehydrated the dried root biomass to examine mycorrhizal colonization. I 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). I 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. I examined root fragments for AMF hyphae, arbuscules, vesicles, as well as for non-AM fungal hyphae. I 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).

Library construction and sequencing

I extracted DNA from roots (~0.15g/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 and Reich 2012. DNA concentrations were determined by PicoGreen (Molecular Probes Inc., Eugene OR, USA) fluorescence and subsequently standardized each sample to ~10 ng/μL.

I 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. (2011). First-round amplifications were carried out with primers possessing universal tails synthesized 5' to the locus specific sequences (Alvarado *et al.* 2017). 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 seconds at 95 °C, 30 seconds at 55 °C, 4 minutes at 60 °C; then refrigerate at 10 °C. I checked the results of the reaction products on a 1% agarose gel. I purified products using a PEG-bead cleanup and eluted in 20 µL Tris-Cl (pH 8.0); I 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 I used 100 nM of each indexing primer, only one reaction was conducted per sample, and only 15 total cycles were performed. I checked indexed PCR products on an agarose gel, and then purified the products with the PEG-bead cleanup, quantified by PicoGreen fluorescence, and pooled equal masses for every sample into a final sample pool using an automated liquid handling system (PerkinElmer, Waltham, MA). I 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 2x300 mode.

Bioinformatics

I joined forward and reverse reads for the ITS 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, I 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. I 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). I extracted fungal sequences from the ITS2 locus using ITSx (Bengtsson-Palme et al. 2013). I picked OTUs using swarm with a resolution of *d4* and assigned taxonomy using BLAST againtst the UNITE database (Kõljalg et al. 2013) for ITS2 and MaarjAM database for 18S (Öpik et al. 2010). OTUs comprising less that 0.005% of the total dataset were removed (Bokulich et al. 2013). OTU tables were rarefied to 14,370 reads for ITS2 and 7,386 reads for 18S for alpha diversity analyses. I 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 http://dx.doi.org/10.17632/gktc62bnhj.1 (Phillips, 2019).

Functional group assignment

To examine responses of the general fungal community (ITS2), I 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, I 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) I assigned families of Glomeromycotina to AMF functional groups: rhizophilic, edaphophilic and ancestral using AMF resource allocation patterns defined in previous studies (Table 2.1, Weber et al. 2019; Phillips et al. 2019).

Statistical analyses

I used the root and shoot biomass (g) data to calculate root to shoot ratios. I 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. I 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. To evaluate whether soil inoculum

source affected root and shoot biomass, I 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, I used a Kruskal-Wallis rank sum test ('kruskal.test' function from the 'stats' package in R). If the Kruskal-Wallis test was significant I 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).

I calculated the alpha diversity for each sample by locus (Chao1, Shannon, and Observed Species) using the core_diversity_analyses.py function in QIIME 1.9.1 (Caporaso et al. 2010). I used the 'kruskal.test' function to determine if there were significant differences in diversity between inoculum source and if significant, I tested the significance of pairwise comparisons using the 'dunnTest' function from the 'FSA' package (Ogle 2018). For each locus, I 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. I 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, I evaluated differences in the OTU richness of the functional groups for both ITS 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).

Indicator Species Analysis

I tested for indicator species/taxa associated with each of the different inoculum treatments for the ITS2 locus. I 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). I only retained taxa with a p value < 0.05 as significant.

Results

Growth response

Adenostoma fasciculatum seedlings grown in either native or invasive inocula had equivalent root:shoot ratios ($p = 0.851$, Figure 3.1A, Supplemental Table 3.1). Seedlings grown with native inoculum had a significantly lower root:shoot ratio than those grown with sterile inoculum ($p = 0.016$, Figure 3.1A, Supplemental Table 3.1). *Adenostoma fasciculatum* seedlings grown with native inoculum produced neither more root nor shoot biomass than those grown with invasive inoculum (Figure 3.1B, $p = 0.237$ and 0.701, respectively). However, seedlings grown with both native and invasive inocula produced

both more root (p = 0.015 and 0.031, respectively) and shoot biomass (Figure 3.1B, p = 0.002 and 0.052, respectively) than those grown with sterile inoculum.

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; Figure 3.2, Supplemental Table 3.2). 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, Figure 3.2, Supplemental Table 3.2).

ITS2 sequences (general fungal community)

I observed a mean ± SE of 2,464 ± 62 reads and 738 ± 25 OTUs per sample. These OTUs belonged to 6 phyla, 15 classes, 40 orders, 68 families and 116 genera. The most abundant phylum in the roots was Ascomycota with 2,225 ± 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 I removed AMF from my analyses, to account for any overlap between my 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.

18S sequences (AMF)

I observed a total of 234 OTUs that were assigned to known taxa after performing BLAST against the MaarjAM database. I observed a mean of 592 $\pm$ 14 (SE) reads, and 120 $\pm$ 3 OTUs, per sample. These OTUs belonged to 4 orders, 9 families and 8 genera within Glomeromycotina. I observed the following 8 genera: *Glomus, Acaulospora, Archaeospora, Paraglomus, Scutellospora, Claroideoglomus, Ambispora,* and *Diversispora*. I placed these OTUs into three functional guilds, as described previously (Table 2.1). Of these functional guilds, the most common guild was rhizophilic AMF families (546 $\pm$ 12 reads and 112 $\pm$ 2 OTUs per sample), followed by ancestral families (50 $\pm$ 0.5 reads and 37 $\pm$ 3 OTUs per sample), with edaphophilic AMF families being the least common (8 $\pm$ 1 reads and 1 $\pm$ 0.05 OTUs per sample) functional guild detected in my study.

Alpha and Beta Diversity

For the ITS2 locus (general fungal community), I detected higher alpha diversity values– Shannon, chao1, and observed species – in roots grown with both native (P = 0.007, 0.0002 and 0.0002, respectively; Supplemental Table 3.3) and invasive (P = 0.031, 0.006 and 0.012, respectively; Supplemental Table 3.3) 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 = 0.923, 0.807 and 0.533, respectively; Supplemental

Table 3.3). For the 18S locus (AMF community), I only observed a difference in Shannon diversity between roots grown with invasive and native inocula sources (P = 0.002; Supplemental Table 3.3) and sterile and native inocula (P = 0.002; Supplemental Table 3.3). Roots grown with native inoculum had higher Shannon diversity values for AMF than those grown with either invasive or sterile inocula sources. General fungal community (ITS2) beta diversity did not differ between inocula types (P = 0.9, Figure 3.3). Additionally, I did not detect any differences in beta diversity of the AMF (18S) community (P = 0.09, Figure 3.4).

Functional Group Responses

General Fungal Community

Non-AMF symbionts 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 = 0.022 and 0.0001; Supplemental Table 3.4; Figure 3.5). Roots grown with invasive inoculum also hosted a richer community of non-AMF symbionts than those grown with sterile inoculum (P = 0.005; Supplemental Table 3.4; Figure 3.5). Although the pathogenic fungal community hosted by roots grown with native inoculum was richer than those grown with sterile inoculum (P = 0.038; Supplemental Table 3.4; Figure 3.5), I did not detect any differences in richness between roots grown with native or invasive inocula (P = 0.533; Supplemental Table 3.4; Figure 3.5). Additionally, there were no detectable differences in pathogen richness between communities hosted by roots grown with invasive and sterile inocula (P = 0.65;

Supplemental Table 3.4; Figure 3.5). The richness of saprotrophs in roots did not differ between inocula (P = 0.416, 0.219 and 0.071; Supplemental Table 3.4, Figure 3.5).

Arbuscular Mycorrhizal Fungal (AMF) Community

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; Supplemental Table 3.4, Figure 3.6). There was no difference in the richness of rhizophillic AMF between roots grown with invasive and sterile inocula (P = 0.936; Supplemental Table 3.4; Figure 3.6). There were no differences in richness of edaphophilic AMF families that primarily produce extraradical or foraging hyphae – between inoculum sources (P = 0.912, 0.521 and 0.988; Supplemental Table 3.4; Figure 3.6). 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; Supplemental Table 3.4; Figure 3.6).

Indicator Species Analysis

Indicator species analysis using the 'multiplatt' function with the indicator value method in the 'indicspecies' R package (Cáceres and Legendre 2009) 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 (Figure 3.7). There were 11 EM species (*Geopora cooperi*, *Choiromyces alveolatus*, *Choiromyces* sp*, Tylospora* sp PG, *Tomentella cinerascens*, *Tuber* sp, *Geopora* sp BS_2010, *Gilkeya compacta*, *Rhodoscypha* sp, *Tuberaceae* sp, *Wilcoxina rehmii*) that had significant indicator values

associated with roots grown with native inoculum, followed by 5 EM species associated with invasive inoculum (Figure 3.7, Supplemental Table 3.5). Two EM species had significant indicator values associated with roots grown in sterile inoculum (Supplemental Table 3.5). Additionally, both *Penicillium brevicompactum* and *Capronia* sp. had significant indicator values associated with native inoculum, whereas *Capronia* sp. was the only endophyte species with a significant indicator value that was associated with invasive inoculum (Supplemental Table 3.5). 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 (Supplemental Table 3.5). One species (*Mastigosporium album*) was a significant indicator value associated with sterile inoculum.

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; Busby et al. 2011; Hausmann and Hawkes 2010; 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). Furthermore, 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. My results show some support for degraded mutualist hypothesis, such that *A. fasciculatum* roots grown with invasive-conditioned soils decreased the richness and abundance of soil symbionts. Likewise, seedlings grown with native inoculum had significantly higher rates of AMF colonization while also hosting 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; Busby et al. 2011; Hawkes et al. 2006).

In addition to hosting a richer community of soil symbionts, there were 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 my 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 observed higher EM richness in roots grown with native inoculum. Additionally, there was lower richness of EM in invasive inoculum and another study has suggested that invasive grass encroachment may decrease EM colonization 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*. In fact, 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).

There were no differences in beta diversity between either the general fungal or AMF communities by inoculum source, likely because inoculum source did not influence edaphophilic AMF and saprotroph richness. There were low richness values of edaphophilic AMF across all inoculum sources which is likely because this study was conducted my 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 there was 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.

Contrary to my findings of low richness of edaphophilic AMF, there was higher overall richness of rhizophilic AMF, consisting of families that primarily colonize roots internally without producing extraradical hyphae and provide protection from pathogenic fungi (Maherali and Klironomos 2007; Sikes et al. 2009). In this greenhouse study, obseved richness of rhizophilic AMF was higher but lower richness of edaphophilic AMF than in the field sampled roots of *A. fasciculatum* (Phillips et al. 2019). Roots grown with native inoculum hosted a richer community of rhizophilic AMF compared to the other inoculum sources which may be due to the greater need for protection from host-specific

pathogens that accumulate in native soils (Sikes et al. 2010, Treseder et al. 2018). Therefore, if native seedlings are propagated in invasive soil, then they are potentially more vulnerable to pathogens, given the reduced presence of rhizophilic AMF; however, it is also possible that there would be a lower abundance of host-specific pathogens in invasive-conditioned soils (Mitchell and Power 2003; Kardol et al. 2007; Van Grunsven et al. 2007; Reinhart et al. 2010). My findings suggest that *A. fasciculatum* seedlings may be more susceptible to pathogens in native soils, because there was higher colonization by non-AMF (which may include pathogens) in roots grown with native inoculum than both those grown with invasive and sterile inocula. Yet, there was no difference in the richness of pathogenic fungi in roots grown with native and invasive inoculum sources. Therefore, native chaparral seedlings may be susceptible to the same pool of fungal pathogens when grown with either native or invasive inoculum, meaning that invasive grasses are not experiencing enemy release. However, native seedlings may 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 my 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 I expected that seedlings grown with native inoculum would produce more biomass than those grown with sterile and invasive inocula, I 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, has 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 I selected *A. fasciculatum*, I 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 this study suggest 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. Overall, the results from this study indicate that out-planting native seedlings cultivated together with native inoculum may improve revegetation success, as these seedlings could serve as nurse plants by creating more favorable microbial communities (Azcón-Aguilar et al. 2003) and promoting chaparral establishment and survival within these threatened ecosystems.

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; Busby et al. 2013). Furthermore, I detected higher rates of AMF and non-AMF colonization in roots grown with native inoculum than those grown with both invasive and sterile inoculum. I did not observe differential growth responses between seedlings grown with either native or invasive inocula, however growth responses may be delayed. Further, this short 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). I conclude that inoculating the soil with live soil inoculum, invasive or native, led to both a more diverse fungal community and a more productive plant community. Overall, these results demonstrate the importance of including soil inoculation along with active restoration techniques when propagating chaparral shrubs to support successful restoration efforts.

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Figures

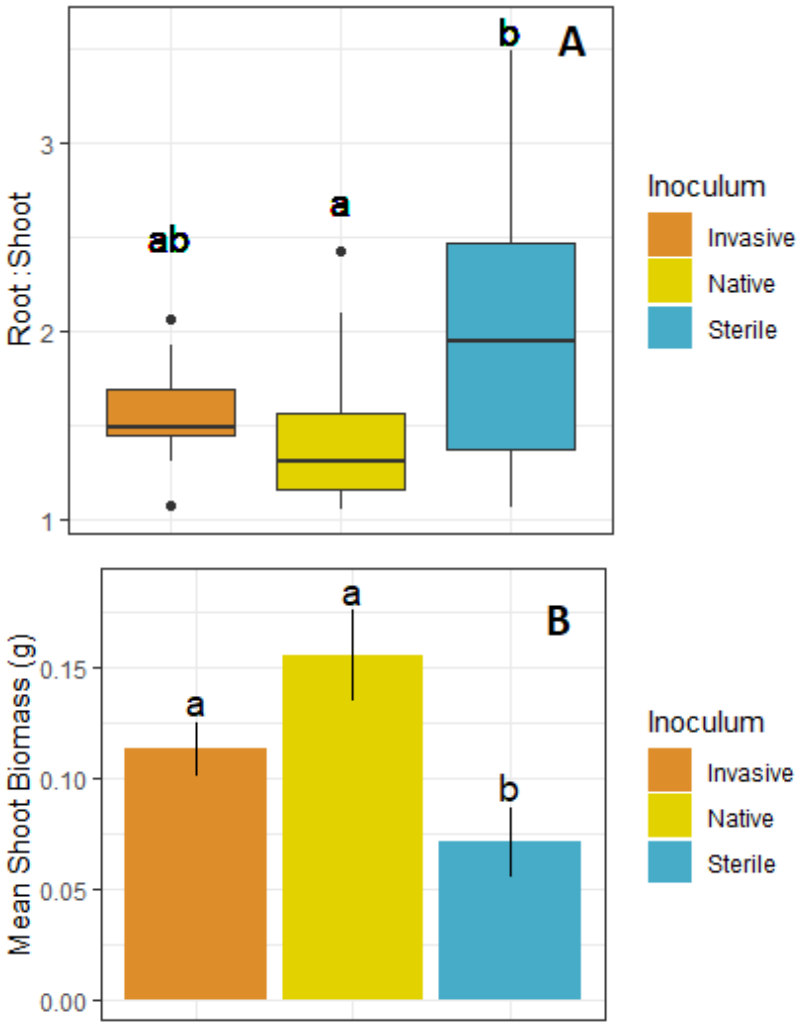

Figure 3.1: (A) Root to shoot ratio and (B) mean shoot biomass (g) of *Adentostoma fasciculatum* seedlings grown with three inoculum types (n = 15) at harvest. Letters indicate significant differences at $p < 0.05$.

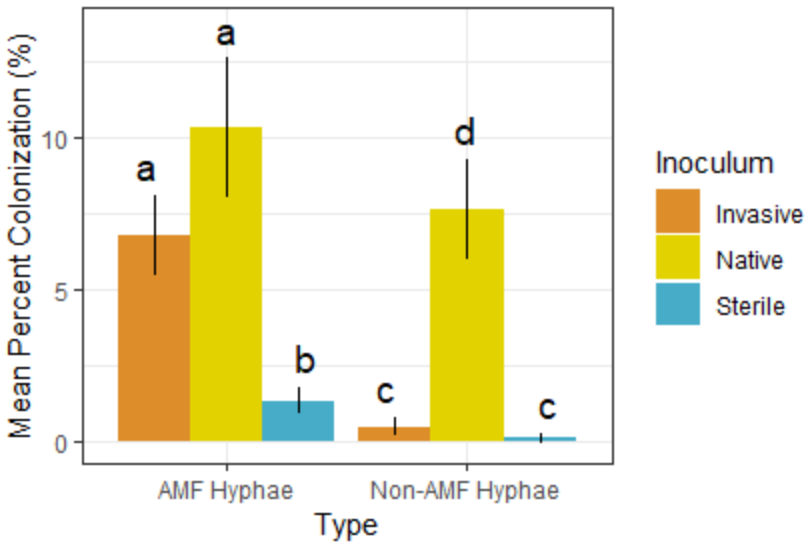

Figure 3.2: Percent colonization of *Adenostoma fasciculatum* roots grown with three inoculum types (n = 15). Letters indicate significant differences at $p < 0.05$.

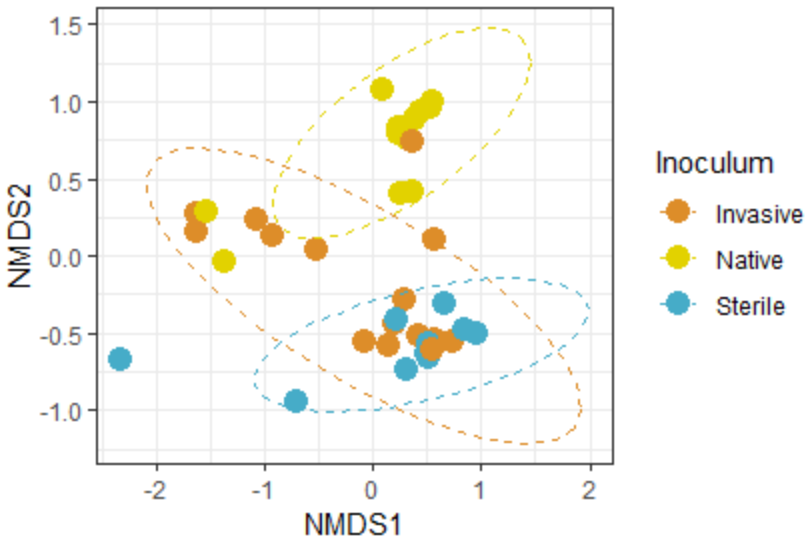

Figure 3.3: General Fungal Community (ITS2) Bray-Curtis NMDS plot. Color is inoculum type (native, invasive, or sterile). The stress value is 0.066.

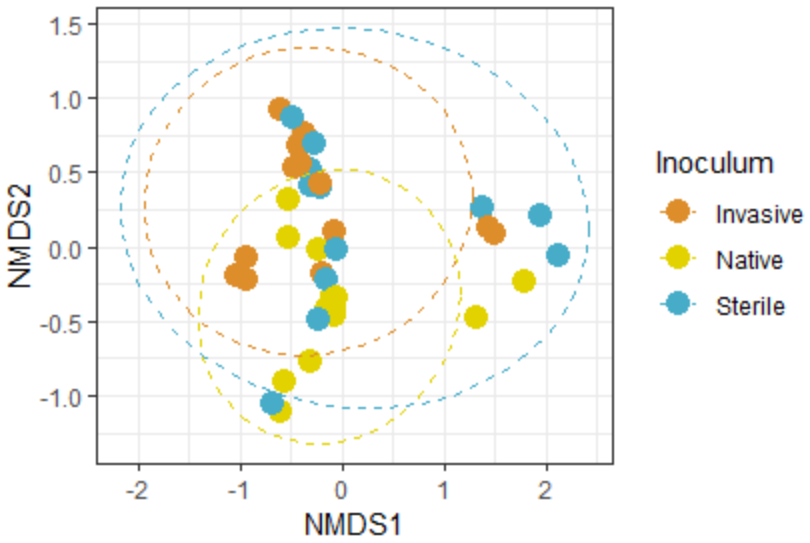

Figure 3.4: AMF (18S) Bray-Curtis NMDS plot. Color is inoculum type (native, invasive, or sterile). The stress value is 0.13.

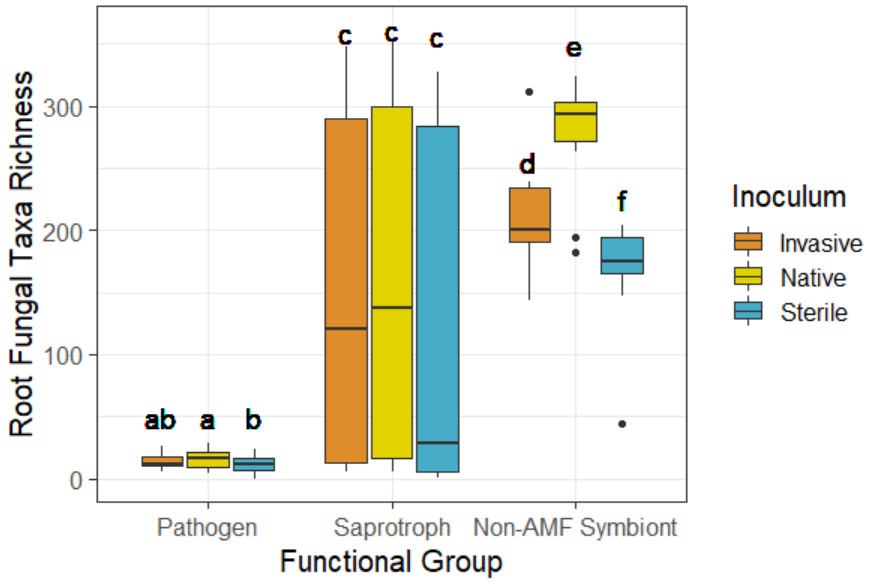

Figure 3.5: ITS2 or general fungal community root community by functional group by aggregating species using FUNguild. Fungal taxa richness is the number of times a unique taxonomic unit is encountered in each sample. Letters indicate significant differences at $p < 0.05$ and can be found in Supplemental Table 3.4.

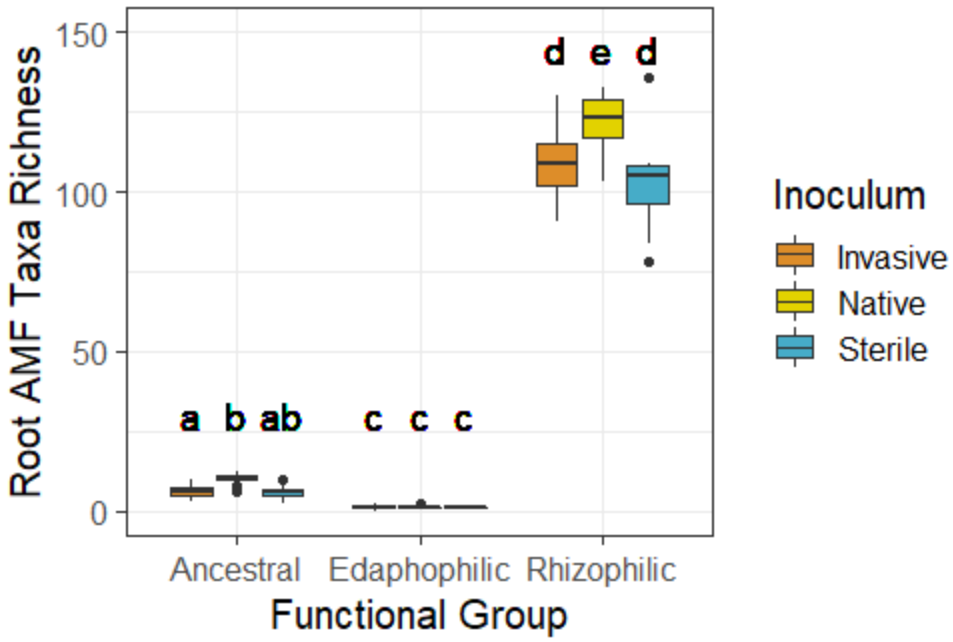

Figure 3.6: 18S or arbuscular mycorrhizal fungal (AMF) root community by functional group. AMF taxa richness is the number of times a unique taxonomic unit is encountered in each sample Letters indicate significant differences at $p < 0.05$ and can be found in Supplemental Table 3.4.

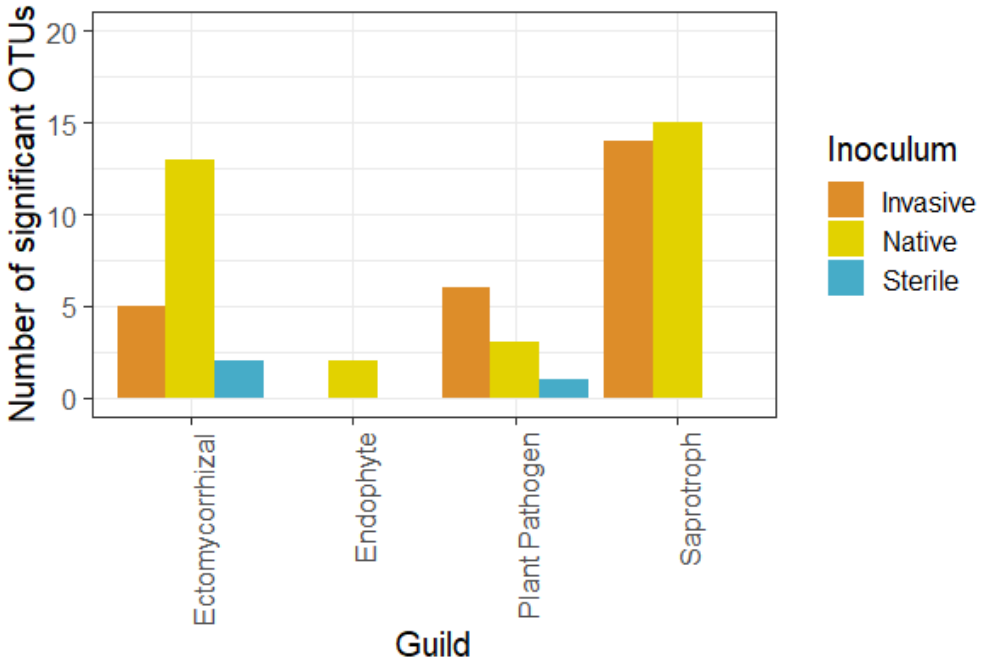

Figure 3.7: Number of OTUs that were significant indicator species for each inoculum type with species grouped by Guilds assign using FUNguild.

Supplemental Tables

Supplemental Table 3.1: P-values from Dunn's Kruskal-Wallis Multiple Comparisons of pairwise comparisons examining differences in biomass production between inoculum types.

Pair-Wise	**Response Variable**	**p-value**
Invasive - Native	Root:Shoot	0.851
Invasive - Sterile	Root:Shoot	0.059
Native - Sterile	Root:Shoot	**0.016**
Invasive - Native	Shoot Biomass	0.237
Invasive - Sterile	Shoot Biomass	**0.052**
Native - Sterile	Shoot Biomass	**0.002**
Invasive - Native	Root Biomass	0.701
Invasive - Sterile	Root Biomass	**0.031**
Native - Sterile	Root Biomass	**0.015**

Supplemental Table 3.2: P-values from Dunn's Kruskal-Wallis Multiple Comparisons of pairwise comparisons examining in differences AMF and non-AMF colonization between inoculum types.

Pair-Wise	**Response Variable**	**p-value**
Invasive – Native	AMF Hyphae	0.8
Invasive - Sterile	AMF Hyphae	**0.013**
Native - Sterile	AMF Hyphae	**0.001**
Invasive – Native	Non-AMF Hyphae	**0.002**
Invasive - Sterile	Non-AMF Hyphae	0.7
Native - Sterile	Non-AMF Hyphae	**0.0003**

Supplemental Table 3.3: P-values from Dunn's Kruskal-Wallis Multiple Comparisons of pairwise comparisons examining differences in alpha diversity metrics between inoculum types.

Pair-Wise	**Alpha-Diversity Metric**	**Locus**	**p-value**
Invasive - Native	Shannon	ITS	0.923
Invasive - Sterile	Shannon	ITS	**0.031**
Native - Sterile	Shannon	ITS	**0.007**
Invasive - Native	Chao	ITS	0.807
Invasive - Sterile	Chao	ITS	**0.006**
Native - Sterile	Chao	ITS	**0.0002**
Invasive - Native	Observed Species	ITS	0.533
Invasive - Sterile	Observed Species	ITS	**0.012**
Native - Sterile	Observed Species	ITS	**0.0002**
Invasive - Native	Shannon	18S	**0.002**
Invasive - Sterile	Shannon	18S	0.933
Native - Sterile	Shannon	18S	**0.002**
Invasive - Native	Chao	18S	0.102
Invasive - Sterile	Chao	18S	0.811
Native - Sterile	Chao	18S	0.943
Invasive - Native	Observed Species	18S	0.072
Invasive - Sterile	Observed Species	18S	0.923
Native - Sterile	Observed Species	18S	0.342

Supplemental Table 3.4: P-values from Dunn's Kruskal-Wallis Multiple Comparisons of pairwise comparisons examining differences in richness of multiple functional groups between inoculum types.

Pair-Wise	Functional Group	Locus	p-value
Invasive - Native	Non-AMF Symbiont	ITS	**0.022**
Invasive - Sterile	Non-AMF Symbiont	ITS	**0.005**
Native - Sterile	Non-AMF Symbiont	ITS	**0.0001**
Invasive - Native	Saprotroph	ITS	0.416
Invasive - Sterile	Saprotroph	ITS	0.219
Native - Sterile	Saprotroph	ITS	0.071
Invasive - Native	Pathogen	ITS	0.533
Invasive - Sterile	Pathogen	ITS	0.625
Native - Sterile	Pathogen	ITS	**0.038**
Invasive - Native	Rhizophilic	18S	**0.037**
Invasive - Sterile	Rhizophilic	18S	0.936
Native - Sterile	Rhizophilic	18S	**0.003**
Invasive - Native	Ancestral	18S	**0.001**
Invasive - Sterile	Ancestral	18S	0.922
Native - Sterile	Ancestral	18S	**0.001**
Invasive - Native	Edaphophilic	18S	0.912
Invasive - Sterile	Edaphophilic	18S	0.521
Native - Sterile	Edaphophilic	18S	0.988

Supplemental Table 3.5: Indicator species analysis results showing species that were significant indicators of each inoculum source.

Guild	Species	Inoculum source(s)	p-value
Ectomycorrhizae	*Choiromyces* sp	Invasive, Native	**0.0003**
Ectomycorrhizae	*Geopora cooperi*	Native	**0.0001**
Ectomycorrhizae	*Choiromyces alveolatus*	Native	**0.0005**
Ectomycorrhizae	*Tylospora* sp PG	Native	**0.0002**
Ectomycorrhizae	*Byssocorticium* sp	Native, Sterile	**0.0372**
Ectomycorrhizae	*Tomentella cinerascens*	Native, Invasive	**0.0107**
Ectomycorrhizae	*Tuber* sp	Native, Invasive	**0.029**
Ectomycorrhizae	*Geopora* sp BS_2010	Native	**0.0001**
Ectomycorrhizae	*Gilkeya compacta*	Native	**0.0022**
Ectomycorrhizae	*Rhodoscypha* sp	Native	**0.0004**
Ectomycorrhizae	*Tuberaceae* sp	Native, Invasive	**0.0003**
Ectomycorrhizae	*Wilcoxina rehmii*	Native	**0.0004**
Endophyte	*Penicillium brevicompactum*	Native	**0.0001**
Endophyte	*Capronia sp*	Native	**0.0001**
Plant Pathogen	*Stagonospora perfecta*	Native	**0.0019**
Plant Pathogen	*Dothiorella brevicollis*	Invasive	**0.0143**
Plant Pathogen	*Mastigosporium album*	Invasive, Sterile	**0.0484**
Plant Pathogen	*Powellomyces* sp	Invasive	**0.0427**
Plant Pathogen	*Lectera longa*	Native, Invasive	**0.0393**
Plant Pathogen	*Pseudofusicoccum kimberleyense*	Native, Invasive	**0.0198**
Plant Pathogen	*Powellomyces hirtus*	Invasive	**0.0019**
Saprotroph	*Talaromyces calidicanius*	Native, Invasive, Sterile	**0.0436**
Saprotroph	*Auricularia fuscosuccinea*	Native	**0.033**
Saprotroph	*Clavaria sp*	Native	**0.0002**
Saprotroph	*Pseudeurotium sp MF_5*	Invasive, Sterile	**0.0127**
Saprotroph	*Phaeococcomyces aloes*	Native, Invasive	**0.0064**
Saprotroph	*Talaromyces amestolkiae*	Sterile	**0.033**
Saprotroph	*Talaromyces ruber*	Native, Invasive, Sterile	**0.0427**
Saprotroph	*Sporormiella sp*	Invasive, Sterile	**0.0018**
Saprotroph	*Lophiostoma sp*	Invasive, Sterile	**0.003**

Saprotroph	*Conocybe aff ochrostriata NL_0830*	Native, Invasive	**0.0003**
Saprotroph	*Ochroconis sp*	Native	**0.03**
Saprotroph	*Mucor velutinosus*	Invasive	**0.0041**
Saprotroph	*Cladophialophora sp*	Native	**0.0007**
Saprotroph	*Talaromyces palmae*	Sterile	**0.035**
Saprotroph	*Lasiosphaeriaceae sp*	Invasive	**0.0253**
Saprotroph	*Talaromyces purpurogenus*	Sterile	**0.022**
Saprotroph	*Auricularia mesenterica*	Native	**0.0002**
Saprotroph	*Polyplosphaeria fusca*	Native	**0.0002**
Saprotroph	*Rhizophlyctis rosea*	Invasive	**0.0402**
Saprotroph	*Davidiella tassiana*	Invasive, Sterile	**0.0111**
Saprotroph	*Auricularia delicata*	Invasive, Native	**0.0104**
Saprotroph	*Dactylella sp*	Invasive	**0.0022**

Chapter 4: Restoring California chaparral: Invasive grass density differentially affects soil water status and native seedling survival

Abstract

Type conversion from native chaparral shrubland to invasive annual grassland is on the rise due to prolonged drought, atmospheric nitrogen deposition and increasing fire frequency. Efforts to restore chaparral ecosystems are limited by current understanding of competitive interaction between shrub seedlings and invasive grasses as well as soil moisture requirements of chaparral seedlings. In this study, I set up a restoration experiment in which I out-planted *Adenostoma fasciculatum* seedlings, manipulated invasive grass density, monitored soil moisture at two depths and tracked seedling survival and biomass. I found that higher invasive grass cover was associated with higher rates of seedling mortality and lower biomass production. In the absence of competition (100% invasive grass removal), I observed higher levels of soil moisture at 35 cm, which may potentially help shrub seedlings persist through the summer drought. Lower invasive cover resulted in higher richness of annual native plant species, as plots with 100% invasive removal had higher richness than 50% removal and unplanted control plots. In sum, 100% invasive grass removal was the most effective treatment in increasing: (1) seedling survival, (2) seedling biomass, (3) soil moisture at 35 cm, and (4) native annual richness. Future restoration efforts in the chaparral should consider invasive grass removal, even if this is labor intensive, to increase initial seedling establishment.

Introduction

Intense prolonged drought episodes (Bell et al. 2004; Dai 2013), atmospheric nitrogen deposition (Fenn et. al 2010) and more frequent fire-return intervals (Keeley and Brennan 2012) are all contributing to the conversion of native vegetation to invasive annual grasses throughout southern California. Chaparral, California's most extensive vegetation type, was historically considered resistant to invasion (Minnich and Bahr 1995; Keeley et al. 2005; Allen et al. 2018), yet has recently undergone invasion because of multiple interacting global change drivers, such as prolonged drought and increased fire frequency (Meng et al. 2014; Dickens and Allen 2014; Stylinski and Allen 1999; Keeley and Brennan 2012). Efforts to restore native plant communities remain limited by our understanding of the mechanisms in which invasive plants outcompete native plants (Funk et al. 2016). To increase our ability to successfully restore native plant communities that provide essential ecosystem services, we need to mechanistically examine how ecological strategies of invasive plants allow them to persist and hamper restoration successes.

Between 2011 and 2016, California experienced a severe drought coupled with record breaking high temperatures (Fahrenkamp-Uppenbrink 2015; Griffin and Anchukaitis 2014), making restoration challenging. Climate-change induced severe drought is linked to mortality of woody vegetation across continents (Allen et al. 2010; Peñuelas et al. 2001). In the most extreme cases, or when prolonged drought is coupled with frequent fire and/or nitrogen deposition, woody native vegetation is replaced by invasive annual grasses. These grasses possess drastically different life-history traits than

the natives they are replacing (Ashbacher and Cleland 2015), such as high-specific leaf area, nitrogen-use efficiency as well as high relative growth rates and seed production (Graebner et al. 2012; Sandel and Dangremond 2012). These fast strategy traits (Wright et al. 2004) contribute to the establishment, especially following disturbance, and persistence of invasive annual grasses while potentially allowing them to outcompete slower-growing native shrubs. To this end, successful restoration in invaded chaparral may rely on understanding resource-use strategies of invasive grasses and how these strategies affect competitive interactions with establishing chaparral shrubs.

Invasive annual grasses escape drought by rapidly using resources and setting seed prior to the summer drought, whereas many chaparral shrubs are evergreen and drought tolerant relying on deep roots to access water during the summer drought (Ackerly 2004). Chaparral shrubs, like other semi-arid plants, specialize in accessing water from different depths of the soil profile during different seasons (Schwinning and Ehleringer 2001; Cody 1986). The strategy of accessing deep water sources during the summer is likely dependent on seasonal precipitation events for recharge through the soil profile. Annual grasses have the potential to deplete soil moisture following precipitation events and thus decrease the amount of available deep-water sources. Chaparral shrubs likely access water in the shallower depths of the soil profile during the rainy season and establishing seedlings will not have fully developed root structures to access deep water, meaning that invasive grasses and chaparral shrubs may have overlapping resource depletion zones making them directly complete for water (Chakraborty and Li 2009).

Efforts to restore plant communities degraded by invasive grasses often focus on removal to decrease competition with natives which requires a large amount of labor but there is limited mechanistic understanding of how invasive removal affects ecosystem function (Eliason and Allen 1997). Furthermore, since type conversion is increasing in the chaparral, active restoration is becoming necessary and few practical strategies have been empirically tested (Allen et al. 2018). Some studies have found that broadcasting seed has not effectively increased establishment (Allen et al. 2018; Stratton 2005) meaning that future efforts will rely on out-planting seedlings grown in nurseries. This study aims to inform future chaparral restoration efforts by increasing understanding of: (1) the effects invasive grasses and their cover have on soil moisture at different parts of the soil profile; (2) how invasive grass presence and cover affects the survival of chaparral seedlings. I present the findings of an eighteen-month restoration experiment in which I tested the response of soil water status and native shrub seedling establishment and growth to multiple levels of invasive species removal. I hypothesize that decreased invasive grass cover via hand removal will increase soil moisture below the rooting zone of the invasive grasses. I also predicted that lower invasive cover would be correlated with higher rates of chaparral seedling biomass production and survival.

Methods

Experimental design

I conducted a field experiment at Emerson Oaks Reserve located in Temecula Valley (33 28’ N, 117 2’ W). The site is dominated by chaparral shrubland in a Mediterranean-type climate with 285 mm average precipitation. There was 508 mm

during the first year (October 2016-September 2017) and 176 mm the second year (October 2017-September 2018). The first year was wetter than average however 90% of the observed precipitation occurred prior to seedling out-planting (between October 2016-February 2017). Much of the reserve burned in a wildfire in 2004 and I set up my experiment in an area where invasive species persisted. These were primarily *Bromus diandrus* and *Bromus rubens* with 100% canopy cover, and <5% understory cover of invasive forbs, primarily *Erodium cicutarium* by late February. In January 2017, I set up twenty $1m^2$ plots with 0.5 m buffers on all sides. I used a randomized block design (blocking by plot location) with five replicate blocks and four treatments: 100% invasive species removal with one shrub seedling planted, 50% invasive removal with one shrub seedling planted, no invasive removal with one shrub seedling planted and a control with no invasive removal and no shrub seedling planted (Figure 4.1). At the time of removal in January invasive annuals were still small and could be removed by hand with minimal soil disturbance. Plots of 50% and 0% cover were maintained by additional invasive removals during the experiment as needed. Most native forbs had not germinated at the time of removal and were left in the plots as they established during the experiment. In all non-control plots, I planted 6-month-old *Adenostoma fasciculatum* seedlings that I grew from seed in 1 L pots in sterilized field collected soil diluted to 50% with sand and 25 g fresh soil as native inoculum collected at the field site. At the time of out-planting, *Adenostoma fasciculatum* seedlings possessed roots approximately 25 cm long. Because of drought, I watered all plots with ~4 liters of water bi-monthly for the first year after transplanting, including unplanted control plots, then ceased watering. Before initial plot

set up, I dug a soil pit to delineate the rooting zone of the invasive grasses. I observed the roots of invasive grasses up to 15 cm at the time of planting.

Vegetation sampling

Monthly, for the entire study period, I measured shrub seedling height and maximum perpendicular diameter (D). I used the following equation to calculate canopy area (A): $A = \pi D^2/4$ (Bonham 1989). Then I calculated shrub volume as a product of the canopy area and shrub height (Bonham 1989). I estimated shrub seedling biomass using an *Adenostoma fasciculatum* species-specific regression equation developed by Vourlitis et al. (2009). I calculated relative growth rate (RGR) for each shrub by dividing the change in biomass between two time points by the number of days between each timepoint. I also measured percent cover of herbaceous/annual vegetation by species and shrub seedling each month starting in year two in each plot. I used the species list from percent cover data to calculate richness of native forbs. I did not include the 0% removal – 1 shrub planted treatment in these measurements, because only one replicate remained alive at the start of year two.

Soil moisture

I dug pits adjacent to each plot to install horizontally integrated soil volumetric water content (VWC) probes underneath planted seedlings (CS-650, Campbell Scientific Inc., Logan, UT) at 15cm and 35cm. I chose these depths to capture soil water status in the grass rooting zone and beneath it. VWC probes were attached to a CR-1000 datalogger (Campbell Scientific Inc., Logan, UT) and measurements were recorded every 15 minutes. I aggregated these measurements into diurnal averages for all downstream

analyses. To understand how each treatment affected soil moisture at 15cm and 35cm, I calculated relative diurnal VWC using the following equation: (treatment - control)/control *100. Control indicates plots where I did not weed or plant a shrub seedling. In the case of seedling mortality in a plot, I removed the VWC from that plot from any mean values of VWC after mortality.

Statistical analyses

I aggregated VWC values across dates to monthly mean VWC values at 15cm and 35cm to match the monthly vegetation sampling, allowing us to incorporate them into models. I built and evaluated three linear mixed effects models using the 'lmer' function from the 'lmerTest' package in R (Kuznetsova et al. 2017), with the following response variables: mean seedling biomass (g), mean VWC at 15 cm, mean VWC at 35cm. The random effect in each model was measurement number, which accounted for the temporal autocorrelation that stems from repeated measures. For model selection, I used the 'step' function from the 'lmerTest' package on full models to do a backwards elimination of fixed effects using AIC. Full model and final model fixed effects for each of the three linear mixed effects models can be found in Table 4.1. Lastly, I built a linear mixed effects model in which native plant richness was the response variable, weeding treatment was the predictor variable and measurement number was the random effect. To asses if invasive removal increased native annual richness, I calculated the estimated marginal means (least-squares means) using the 'emmeans' function with Tukey's adjustment from the 'emmeans' package in R (Lenth 2019).

Results

Seedling mortality

In May 2017, four months after out-planting, two replicate shrub seedlings in 0% invasive removal plots died and one replicate shrub seedling in a 50% invasive removal plot died (Figure 4.2). In September 2017, eight months after out-planting, two additional shrub seedlings in 0% removal plots died and an additional replicate shrub seedling in a 50% removal plot died (Figure 4.2). This left only one replicate seedling remaining alive in the 0% removal plots and three replicate seedlings in the 50% removal plots. I replaced the two replicates in the 50% removal plots in January 2018 with extra shrub seedlings I reserved and hardened off at the time of out-planting (Figure 4.2). However, both shrub seedlings that I transplanted to the 50% removal plots died in September 2018 (Figure 4.2). I decided not to replace the four replicates in the 0% invasive removal plots, since they experienced 80% mortality within the first 8 months of the experiment. The plots with 100% invasive removal did not experience any mortality.

Relative growth rate and seedling biomass

Relative growth rate calculations indicate that seedlings primarily produced biomass in the spring (Figure 4.3). However, during the second year of the study seedlings maintained a mean RGR above 0.5 into May. Plots with 50% removal had a negative mean RGR in the spring 2018 when grasses were active but had a slightly positive mean RGR in June 2018 after grasses senesced (Figure 4.3). Standard error was high for mean RGR in the 50% weeded plots during Spring 2018, which may be due to the two new transplants in January replacing those that died in 2017. RGR for the 0%

weeded plots was only included until September 2017, when only one replicate remained alive. Mean RGR for the seedlings in the 0% weeded plots was slightly positive until September 2017 but was also much lower than the other treatments in the months immediately following transplant (Figure 4.3).

Weeding treatment was correlated with mean seedling biomass, as seedlings planted in the 100% and 50% invasive removal plots produced more biomass than the 0% removal plots ($p = 0.003$ and 0.05, respectively; Figure 4.2; Table 4.1 and Supplemental Table 4.1). Mean VWC at 15cm and 35cm were also positively correlated with mean seedling biomass ($p = 0.0124$ and 0.0099, respectively; $R^2 = 0.84$; Supplemental Table 4.1). Lastly, the interaction between mean VWC at 15 cm and mean VWC at 35 cm was negatively corelated with mean seedling biomass ($p = 0.0034$; Supplemental Table 4.1; $R^2 = 0.84$).

Precipitation and soil moisture

Control and 100% weeded plots had higher soil moisture within the grass rooting zone (15 cm) during fall 2017 and January 2018 than in the 50% weeded plots (Figure 4.4A). Diurnal VWC at 15cm at was steadily higher than the control and other treatments in plots with 50% removal (Figure 4.4A). There were numerous rain events during spring 2018, with some of them being quite large, which led to highest soil moisture values at 15cm in plots with 50% removal (Figure 4.4A and 4.5A). Grasses had established by this time (grasses germinate in late January 2018). In contrast, at 35 cm, below the rooting zone of the grasses, both control and 100% removal had higher soil moisture than the 50% removal plots (Figure 4.4B and 4.5B).

Monthly mean VWC at 15cm was positively correlated with 50% invasive grass removal ($p = 0.00001$; $R^2 = 0.66$; Table 4.1 and Supplemental Table 4.2). Mean seedling biomass was negatively correlated with mean VWC at 15cm ($p = 0.004$; $R^2 = 0.66$; Table 4.1 and Supplemental Table 4.2). Additionally, the interaction between 50% removal treatment and mean VWC at 35 cm as well as the interaction between mean seedling biomass and mean VWC at 35 cm were negatively correlated with mean VWC at 15cm ($p = 0.00001$ and 0.01, respectively; $R^2 = 0.66$; Table 4.1 and Supplemental Table 4.2).

Monthly mean VWC at 35cm was positively correlated with both 50% and 100% invasive removal ($p = 0.0002$ and 0.0252, respectively; $R^2 = 0.78$; Table 4.1 and Supplemental Table 4.3). The interaction between the 50% removal treatment and mean VWC at 15 cm and the interaction between the 50% removal treatment and seedling biomass were negatively correlated with mean VWC at 35 cm ($p = 0.01$ and 0.03, respectively; $R^2 = 0.78$; Table 4.1 and Supplemental Table 4.3). Lastly, mean VWC at 15 cm was positively correlated with mean VWC at 35 cm ($p = 0.007$; $R^2 = 0.78$; Table 4.1 and Supplemental Table 4.3).

Native species richness

In 100% removal plots, bare ground cover was an average of 87% $\pm$ 8.4% (mean $\pm$ standard deviation. In 50 and 100% removal plots, bare ground cover was 60% $\pm$ 17.5% and 9.7 $\pm$ 6 %, respectively. Plots with 100% invasive removal had higher native species richness than 50% removal and control plots ($p = 0.0001$ and 0.0001, respectively; $R^2 = 0.49$; Supplemental Table 4.4; Figure 4.6). There was no difference in native species richness between 50% weeded and control plots ($p = 0.41$, $R^2 = 0.49$

Supplemental Table 4.4; Figure 4.6). However, cover of native annuals was always < 10% of each plot.

Discussion

My results demonstrate that invasive grass removal likely contributes to both native seedling growth and survival as well as increased soil water availability at 25 cm. Higher invasive cover led to increased mortality, 80% mortality in plots with no removal and 40% mortality in plots with 50% invasive removal, where plots with all invasives removed experienced 0% mortality. Additionally, decreases in invasive cover were correlated with increases in shrub seedling biomass. Soil moisture was often highest in 50% removal plots at 15cm, which may be due to overlapping rooting zones of invasive grasses and native seedlings. However, at 35 cm soil moisture was often higher in treatments without competition between natives and invasive (control and 100% invasive removal). During year two of my study, soil moisture at 35cm was consistently the highest in 100% invasive removal plots, suggesting that increasing invasive grass cover depletes moisture, promoting competition between native and invasive species. This work supports the hypothesis that shallow-rooted invasive annual grasses rapidly deplete soil moisture in the upper parts of the soil profile, thus negatively affecting deeper rooted perennial shrubs (Eliason and Allen 1997; Wood et al. 2006; DeFalco et al. 2007).

As invasive density increased in my treatments, so did seedling mortality, and more specifically in plots with no invasive removal, as 80% of seedlings died within eight months of out planting. Mortality did not occur until severe soil drying in summer. These mortalities may be a consequence of a seasonal priority effect – through earlier

phenological activity – conferred to the invasive annual grasses (Young et al. 2001; Wolkovich and Cleland 2011; Wainwright et al. 2012). The potential of invasive grasses to gain seasonal priority and outcompete chaparral shrub seedlings is particularly important to consider in post-disturbance restoration efforts.

Passive restoration is often the most straightforward management option and has garnered success in some systems (De Steven et al. 2010; Holl and Aide 2011), but it has proven to be an ineffective approach in the chaparral, especially following disturbance, due to rapid colonization of invasive grasses (Corbin and D'Antonio 2004). Additionally, active restoration attempts via broadcasting seed have also been unsuccessful (Allen et al. 2018; Stratton 2005). These results add to this understanding of chaparral restoration, in that out-planting seedlings without further invasive removal resulted in chaparral seedling establishment. However, removal coupled with out-planting not only increased shrub survival but also led to both greater increases in shrub biomass and richness of native annual plants. With increases in fire-return intervals a large proportion of restoration efforts are in invaded areas (Allen et al. 2018). My results suggest that, while cost and labor intensive, invasive removal during the first year of restoration can greatly improve seedling survival as well as biomass production. Additionally, establishment of native chaparral seedlings have the potential to shift the successional trajectory of invaded communities by altering resource availability belowground (i.e. water and nutrients) as well as fostering a more favorable microbial community and thus initially established plants may act as nurse plants (Azcón-Aguilar et al. 2003).

My results support the hypothesis that various levels of invasive grass cover would differentially affect soil water availability both within and below the invasive grass rhizosphere. It is important to note that while I observed grass roots extending to 15 cm, other studies have observed invasive grass roots extending much deeper (~35 cm), meaning that later in the spring grass roots may have been active at 35 cm (Eliason and Allen 1997). However, the dominant exotic grass at their site was *Avena* spp, which is more robust than *B. diandrus*, the dominant at my site, and precipitation was double average during their measurements (Eliason and Allen 1997). At the shallow depth (15 cm), soil moisture was often higher in the 50% invasive removal plots than both the control and 100% invasive removal plots. It is likely that the 100% removal plots had higher rates of runoff following precipitation events since there was a greater area of bare ground (Figure 4.1). Differences in soil moisture at 15 cm between 50% invasive removal and 100% invasive removal were the most pronounced during the spring, when grasses are active and likely when chaparral shrubs will access water shallower in the soil profile (Phillips Chapter 1; Schwinning and Ehleringer 2001; Cody 1986). This competition for water at 15 cm and a more rapid depletion of available moisture by invasive grasses (DeFalco et al. 2007) may also contribute to my finding that soil moisture was greater in the 100% removal plots than 50% removal plots below the rooting zone of the invasive grasses (35 cm). This suggests that in the absence of competition there is a higher propensity for soil water recharge within the soil profile. Furthermore, soil moisture at 35cm in the 100% removal plots was steadily higher in the late spring and early summer

which could contribute to greater seedling survival than in the 50% and 0% invasive removal.

The differences in soil moisture at both depths between treatments were correlated to *A. fasciculatum* seedling biomass, suggesting that moisture requirements strongly affect successful establishment of chaparral shrubs. I found some evidence that moisture requirements vary with shrub age, as the interaction between soil moisture at 15 cm with soil moisture at 35 cm was positively correlated with seedling biomass. This suggests that the relationship between soil moisture at 15 cm and 35 cm is less important as the shrubs get larger, which might be because as the shrubs get larger, they are less dependent on shallow soil moisture and more dependent on deeper moisture. Drought tolerance of many chaparral shrubs develops with age likely in parallel with root development, yet our understanding of chaparral seedling drought tolerance is poor (Allen et al. 2018). The little information available about chaparral seedling drought tolerance suggests high rates of seedling mortality (> 90%) during post-fire succession, which may be due in part to competition with invasive grasses as well as a lack of shade (Kummerow et al. 1985; Moreno and Oechel 1992; Pratt et al. 2008). This study was not conducted immediately post-fire, but rather in a grass-invaded site that might pose even more moisture stress on seedlings establishment.

Conclusions

Increasing invasive cover was associated with increased seedling mortality and lower seedling biomass. I observed higher mortality in the 50% invasive removal (40%) than the 100% invasive removal (0%) plots, but I also observed higher soil moisture at

15cm in the 50% invasive removal plots. While I observed 40% mortality in the 50% invasive removal, this was lower than mortality in the 0% invasive removal (80%) and seedlings in these plots produced more biomass than those in the 0% removal plots. Therefore, these results suggest that weeding is effective in increasing seedling establishment and biomass production. Differences in soil moisture at both depths between treatments were correlated to *A. fasciculatum* seedling biomass, suggesting that moisture requirements strongly affect successful establishment of chaparral shrubs. Furthermore, higher amounts of soil moisture at 35 cm in plots with 100% removal which has the potential to increase the likelihood that seedlings could survive the intense summer drought period. This also suggests that when established these seedlings could facilitate the establishment of other shrub seedlings (Keeley 1992; Azcón-Aguilar et al. 2003; Pratt et al. 2008). While 50% invasive removal did increase the survivorship of seedlings compared to no removal treatments, the benefits conferred from 100% removal were greater from multiple perspectives: (1) higher seedling biomass; (2) no seedling mortality; (3) significantly higher annual richness; (4) higher rates of soil moisture at 35cm, especially in the late summer. This study takes the first step in understanding the soil moisture requirements for successful chaparral seedling establishment and demonstrates that while cost- and labor-intensive removal is a crucial component of initial steps to restore type-converted or recently disturbed chaparral ecosystems.

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Tables

Table 4.1: Response variables for the three linear mixed effects models with candidate fixed effects (or predictor variables) and fixed effects retained in each of the final models after backwards elimination using AIC. The fixed effect weeding treatment had three levels: 0% removal, 50% removal and 100% removal). The random effect in each model was measurement number to account for temporal autocorrelation in the repeated measures analysis.

Response Variable	Candidate Fixed Effects	Retained Fixed Effects	R^2 for final model
Mean Seedling Biomass	(1) weeding treatment, (2) monthly mean VWC at 15 cm (3) monthly mean VWC at 35 cm (4) weeding treatment X monthly mean VWC at 15 cm (5) weeding treatment X monthly mean VWC at 35 cm (6) monthly mean VWC at 15 cm X mean VWC at 35 cm	(1) weeding treatment, (2) monthly mean VWC at 15 cm (3) monthly mean VWC at 35 cm (4) monthly mean VWC at 15 cm X mean VWC at 35 cm	0.84
Monthly Mean VWC at 15 cm	(1) weeding treatment, (2) mean seedling biomass (3) monthly mean VWC at 35 cm (4) weeding treatment X mean seedling biomass (5) weeding treatment X monthly mean VWC at 35 cm	(1) weeding treatment, (2) mean seedling biomass (3) monthly mean VWC at 35 cm (4) weeding treatment X mean seedling biomass (5) weeding treatment X monthly mean VWC at 35 cm	0.66
Monthly Mean VWC at 35 cm	(1) weeding treatment, (2) mean seedling biomass (3) monthly mean VWC at 15 cm (4) weeding treatment X mean seedling biomass (5) weeding treatment X monthly mean VWC at 15 cm	(1) weeding treatment, (2) mean seedling biomass (3) monthly mean VWC at 15 cm (4) weeding treatment X mean seedling biomass (5) weeding treatment X monthly mean VWC at 15 cm	0.78

Figures

Figure 4.1: Photos of field plot treatments: (A) 100% invasive removal with *Adenostoma fasciculatum* shrub (center) and colonizing native annuals, (B) 50% invasive removal (shrub seedling in red circle) and (C) 0% invasive removal.

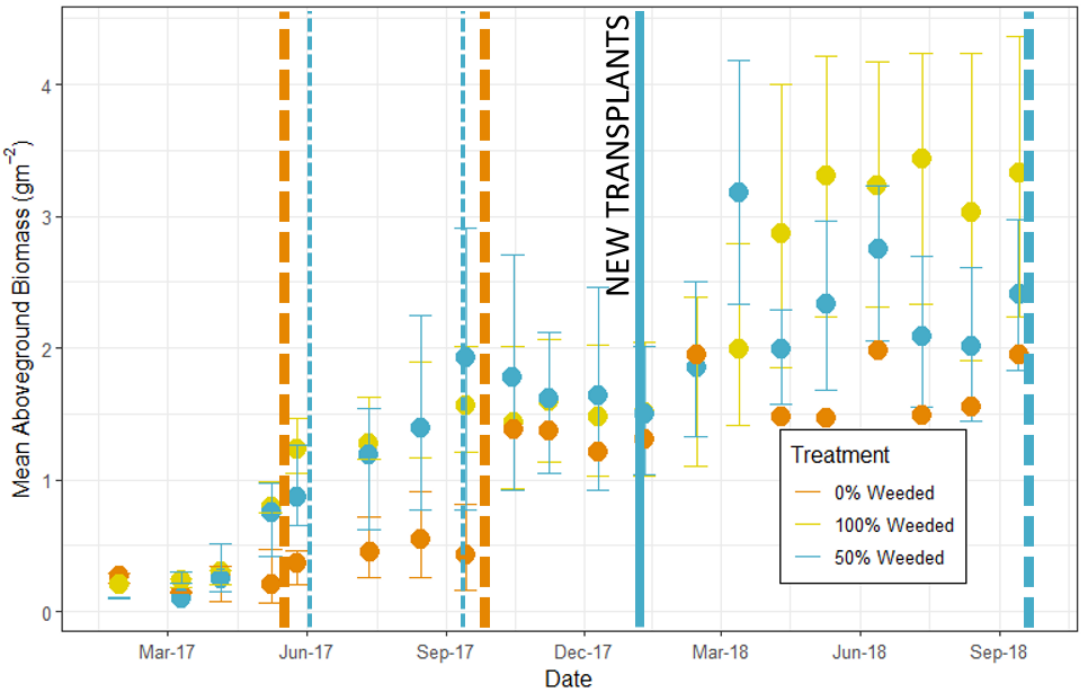

Figure 4.2: Mean shrub seedling biomass (points; n = 5) with standard error. Bold dashed lines indicate mortality of two seedlings, color indicates treatment. Thinner dashed line indicates mortality of one seedling, color indicates treatment. Solid line represents transplants in 50% weeded plots to replace the two mortalities. Plots with no weeding experienced 80% mortality, standard error is not included after September 2017, because only one replicate remains. Plots with 50% invasive removal experienced 40% mortality. Plots with 100% invasive removal experienced 0% seedling mortality.

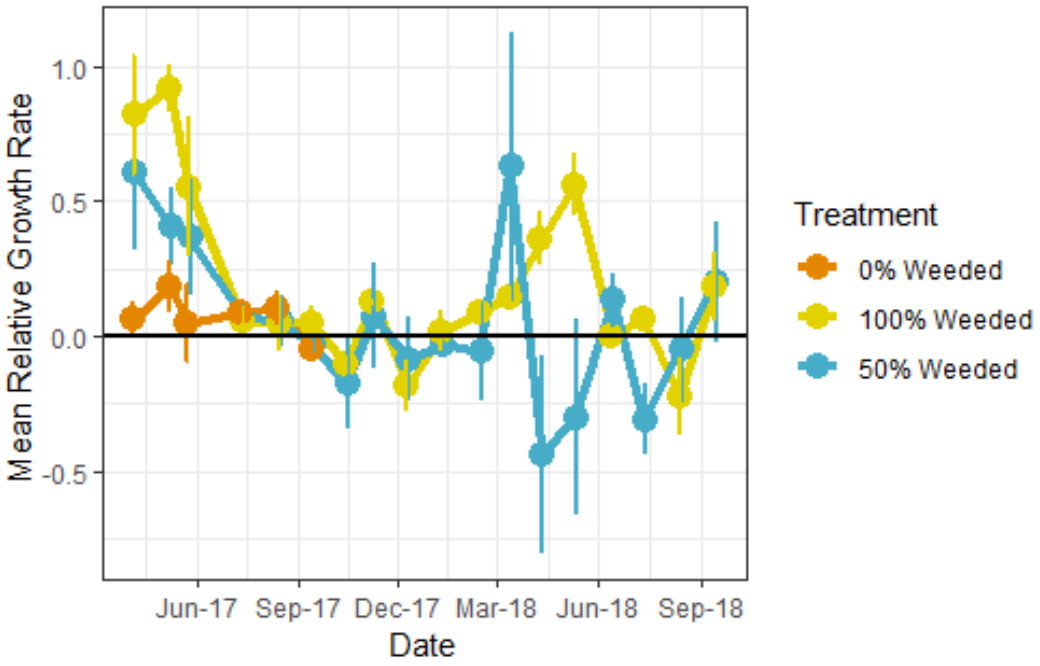

Figure 4.3: Mean relative growth rate using biomass measurements with standard error (vertical lines) through time of shrub seedlings by weeding treatment (n = 5). Negative values represent stem dieback. We did not include RGR values for 0% weeded (orange) after September 2017 because only one replicate survived.

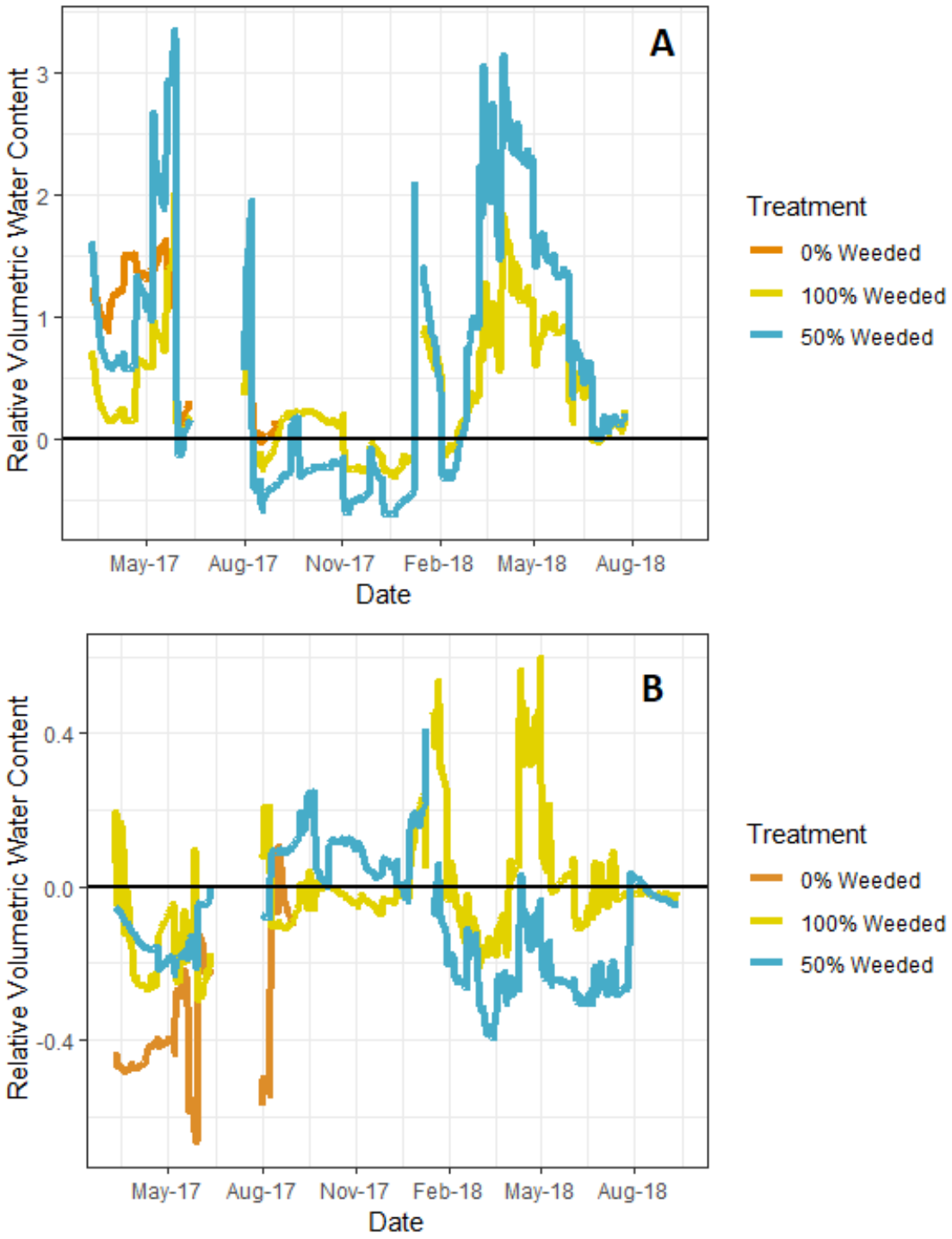

Figure 4.4: Relative volumetric water content by weeding treatment (n = 5) at 15cm (A) and 35cm (B). We removed data from 0% weeded plots after 80% of seedling mortality in September 2017. Relative VWC is calculated using this formula: (treatment - control)/control *100.

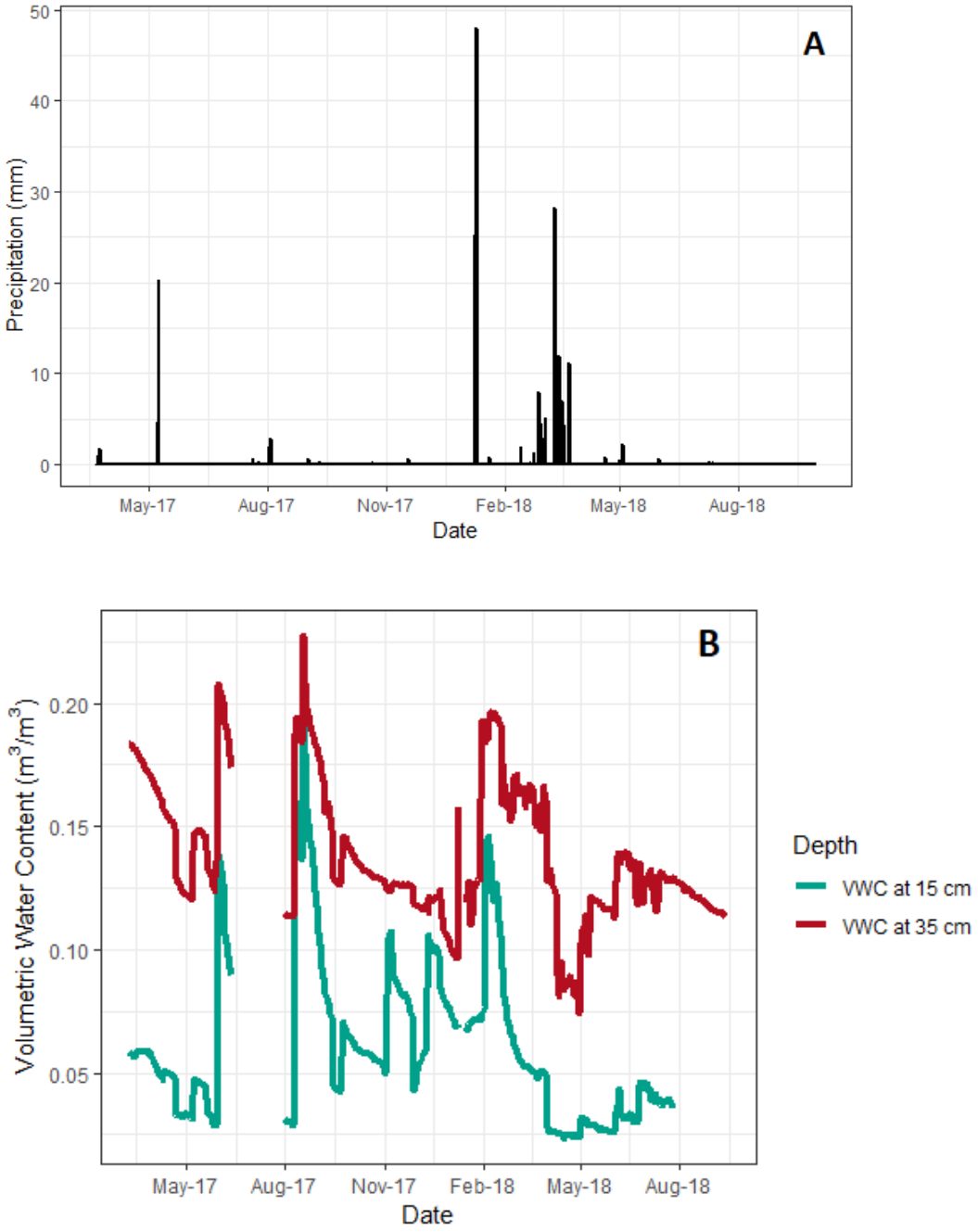

Figure 4.5: Daily precipitation (mm) for entire study period (A). Diurnal volumetric water content (m^3/m^3) by weeding treatment (n = 5) at 15cm and 35cm for control plots (no weeding and no shrub seedling planted). Gaps in data (B) are due to power failure.

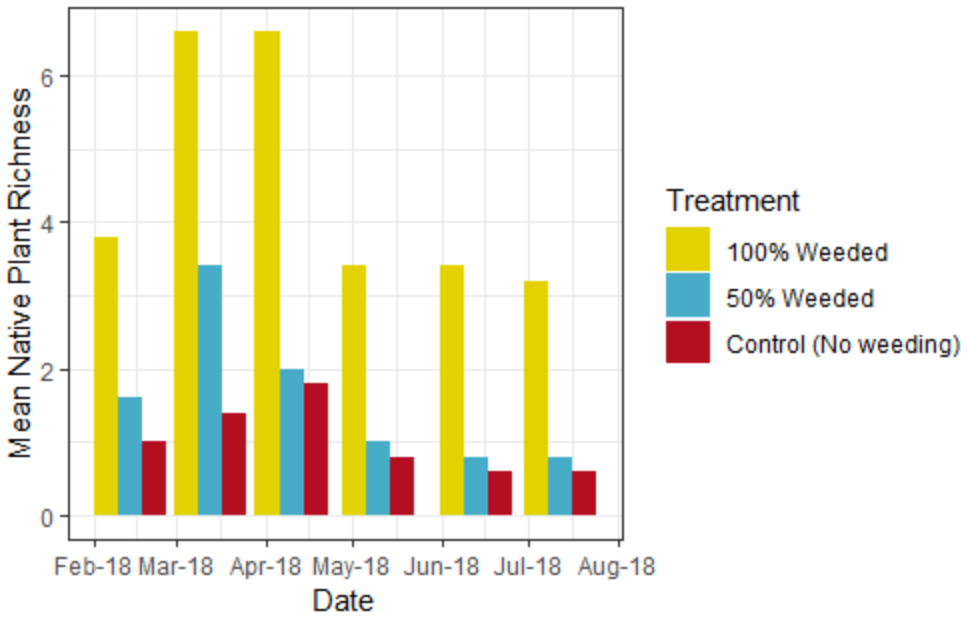

Figure 4.6: Mean annual native plant richness within 1m^2 plots (n =5) at each sampling data. We did not include the 0% weeding because only one replicate remained.

Supplemental Tables

Supplemental Table 4.1: Results of linear mixed effects model examining effects of weeding treatment on shrub seedling biomass. Fixed effects were: weeding treatment, mean VWC at 15 cm, mean VWC at 35 cm, and mean VWC at 15 cm: mean VWC at 35 cm. Measurement was the random effect to account for temporal autocorrelation of data. Significant p-values are bold. The lmer function automatically calculates t-tests using Satterthwaite approximations to degrees of freedom.

Fixed Effects	Estimate	Standard Error	df	t value	Pr(>|t|)
Weeding treatment - 100% removal	0.6206	0.1970	36	3.1509	**0.0033**
Weeding treatment - 50% removal	0.3637	0.1829	35	1.9889	**0.0544**
Mean VWC at 15 cm	16.0666	6.0375	30	2.6611	**0.0124**
Mean VWC at 35 cm	14.3147	5.2227	32	2.7409	**0.0099**
Mean VWC at 15 cm: mean VWC at 35 cm	-175.5838	55.4994	33	-3.1637	**0.0034**

Supplemental Table 4.2: Linear mixed effects model outputs of response of soil VWC at 15 cm. Fixed effects were: weeding treatment, mean seedling biomass, mean VWC at 35 cm, weeding treatment: mean VWC at 35 cm, and mean seedling biomass: mean VWC at 35 cm. Measurement was the random effect to account for temporal autocorrelation of data. Significant p-values are bold. The lmer function automatically calculates t-tests using Satterthwaite approximations to degrees of freedom.

Fixed Effects	Estimate	Standard Error	df	t value	Pr(>|t|)
Weeding treatment - 100% removal	0.0546	0.0438	40	1.2467	0.2198
Weeding treatment - 50% removal	0.1528	0.0304	32	5.0189	**0.0001**
Mean seedling biomass	-0.0743	0.0242	44	-3.0697	**0.0037**
Mean VWC at 35 cm	0.3190	0.3084	42	1.0344	0.3069
Weeding treatment- 100%: Mean VWC at 35 cm	-0.5983	0.3291	35	-1.8180	0.0776
Weeding treatment- 50%: Mean VWC at 35 cm	-1.3056	0.2506	30	-5.2104	**0.0001**
Mean seedling biomass: Mean VWC at 35 cm	0.5060	0.1876	41	2.6981	**0.0101**

Supplemental Table 4.3: Linear mixed effects model outputs of response of soil VWC at 35 cm. Fixed effects were: weeding treatment, mean seedling biomass, mean VWC at 15 cm, weeding treatment: mean seedling biomass, and weeding treatment: mean VWC at 15 cm. Measurement was the random effect to account for temporal autocorrelation of data. Significant p-values are bold. The lmer function automatically calculates t-tests using Satterthwaite approximations to degrees of freedom.

Fixed Effects	Estimate	Standard Error	df	t value	Pr(>|t|)
Weeding treatment - 100% removal	0.0666	0.0278	23	2.3955	**0.0252**
Weeding treatment - 50% removal	0.1117	0.0261	25	4.2825	**0.0002**
Mean seedling biomass	0.0123	0.0114	30	1.0808	0.2885
Mean VWC at 15 cm	0.4581	0.1593	31	2.8754	**0.0072**
Weeding treatment-100%: mean seedling biomass	-0.0101	0.0116	24	-0.8704	0.3928
Weeding treatment-50%: mean seedling biomass	-0.0279	0.0120	24	-2.3238	**0.0289**
Weeding treatment-100%: mean VWC at 15 cm	-0.2691	0.2297	23	-1.1714	0.2533
Weeding treatment-50%: mean VWC at 15 cm	-0.5289	0.1908	24	-2.7720	**0.0106**

Supplemental Table 4.4: Outputs from estimated marginal means (least-squares means), with native plant richness as the response variable. Direction of effect indicated the effect the first term in the pairwise comparison had on native plant richness compared to the second term.

Pairwise comparison	p-value	Direction of effect
100% removal – 50% removal	**0.0001**	+
100% removal – Control	**0.0001**	+
50% removal – Control	0.4124	+

Synthesis and Future Directions

This dissertation research is focused on understanding the mechanisms that allow invasive grasses to persist in areas that were formerly dominated by chaparral vegetation. Historically, chaparral has been resilient to disturbance, but in recent years accelerating fire frequency coupled with anthropogenic nitrogen deposition are contributing to invasion in the chaparral (Stylinski and Allen 1999; Keeley and Brennan 2012; Meng et al. 2014; Dickens and Allen 2014). Invasion often leads to vegetation community type conversion, in this case chaparral shrubland to invasive grassland, which are often accompanied by changes in resource availability and soil microbial communities (Hawkes et al. 2006). As type conversion of chaparral shrublands rises, so does interest in restoring chaparral plant communities (Allen et al. 2018), yet efforts to restore these native plant communities remain limited by which invasive plants compete with native plants (Funk et al. 2016).

To examine multiple mechanisms that may contribute to invasive persistence, I integrated tools and perspectives from community and ecosystem ecology. In Chapter 1, I examined how rooting and water-use strategies differ between a native chaparral shrub and an invasive grass and found that the invasive grass would both produce longer roots at shallow depths and deplete soil moisture earlier in the growing season than the native shrub. Furthermore, these results suggest invasive grasses have the potential to accelerate the onset of the summer drought and decrease deep soil water recharge. This could inhibit the re-establishment of native shrubs and further increase vulnerability to invasion, however further investigation of invasive grass water-use at multiple sites and in mixed

stands are needed. In Chapters 2 and 3, I aimed to understand how invasion restructures soil fungal communities and how changes in composition subsequently affect native seedling growth. My results suggest that invasion decreases the presence of multiple groups of mycorrhizal fungi, demonstrating the necessity of considering soil microbial communities into future restoration efforts. Chapter 4 takes the first step in understanding the soil moisture requirements for chaparral seedling establishment and demonstrates that while cost- and labor-intensive, invasive grass removal is a crucial component of initial steps to restore type-converted or recently disturbed chaparral ecosystems.

Restoration efforts in the chaparral will likely depend on nursery-grown transplants as seeding efforts have had poor success (Stratton 2005; Allen *et al.* 2018), making it imperative to understand effects of plant-soil feedbacks on chaparral seedlings. Previous research in other systems has established that invasive annual grasses can benefit from the *a priori* presence of symbiotic fungi, absence of host-specific fungal pathogens, and/or by altering the microbial community (Reinhart and Callaway 2006; Pringle et al. 2009; van der Heijden et al. 2008; Hilbig and Allen 2015). Chapter 2 illustrates that invasive grasses altered soil fungal communities, as invasive grass roots hosted less rich and abundant communities of both AMF and non-AMF symbionts compared to native shrubs. This suggests 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; Busby et al. 2013). I found further support for this idea in Chapter 3, where chaparral seedlings grown with native inoculum hosted a richer community of fungal symbionts than those grown with invasive and

sterile inoculum. While seedlings grown with native inoculum hosted a richer community of fungal symbionts, they did not have a greater growth response than those grown with invasive inoculum. Yet, this was a relatively short (6 months) greenhouse study and others have found that native inoculum may increase establishment when transplanted to type-converted field sites (Middleton and Bever 2012; Wubs et al. 2016). Therefore, future efforts to understand the effects of microbial soil legacies on native re-establishment in the chaparral would benefit from monitoring microbial communities after out-planting to the field. Also, the plant community restoration literature, especially regarding semi-arid ecosystems, would greatly benefit from a broader investigation of more native and invasive plant species interactions with soil microbes.

Unlike mature chaparral shrubs, seedlings have yet to develop the extensive root structures that allow mature shrubs to access deep water sources, which means that chaparral seedlings and invasive annual grasses will have overlapping resource depletion zones and potentially directly compete for water (Chakraborty and Li 2009). I found that higher invasive cover resulted in higher shrub seedling mortality rates and that soil moisture at both depths (15 cm and 35 cm) were correlated with seedling biomass. Additionally, soil moisture at 35 cm was highest in the 100% removal plots which could help these seedlings survive the summer drought. Furthermore, if established these seedlings could potentially facilitate the establishment of other shrub seedlings by creating more favorable soil conditions – both through increased soil moisture and presence of mycorrhizal fungi (Keeley 1992; Azcón-Aguilar et al., 2003; Pratt et al. 2008).

Overall, my dissertation suggests that both competition between invasive and native plants as well as shifts in fungal communities help invasive grasses to persist. The decrease in multiple groups of mycorrhizal symbionts coupled with higher mortality rates in plots with greater invasive cover, suggests that successful restoration of the chaparral will need to include both native inoculation and invasive grass removal in the initial establishment phases. Furthermore, future research should continue to monitor the fungal communities colonizing native roots once out-planted to assess whether the presence of host-specific mutualists can facilitate the establishment of native shrubs (Johnson et al. 2009). This work adds to a growing body of evidence that active restoration is necessary to restore chaparral plant communities (Allen et al., 2018; Stratton 2005), while also highlighting the importance of including linkages between aboveground plant communities and belowground microbial communities.

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