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Ecological Assembly Rules and Soil Legacy Effects in the Restoration of an Invaded Plant Community

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ABSTRACT OF THE DISSERTATION

Ecological Assembly Rules and Soil Legacy Effects in the Restoration of an Invaded Plant Community

by

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Understanding the composition of ecological communities that arise from potential species pools has implications for community assembly and applications for restoration. Invasive species pose special challenges to restoration by contributing to ecosystem degradation as well as resisting restoration efforts. In the face of such challenges, understanding the complex of mechanisms working together to enable an invasive species to establish and spread may lead to better management strategies and greater restoration success. The overall objective of this dissertation is to understand mechanisms contributing to the success of a Mediterranean annual grass, *Bromus diandrus*, through the use of both field and greenhouse studies, and to use this understanding to inform restoration of invaded ecosystems. More specifically, I consider three potential mechanisms of invasion: 1) plant functional traits, 2) plant-soil feedback, and 3) soil legacy effects. The results of the studies of this dissertation demonstrate that multiple mechanisms of invasion promote *Bromus diandrus* success. First, functionally

similar native plant communities did not demonstrate biotic resistance to *B. diandrus* invasion during restoration studies. Rather, earlier germination and larger seed mass of *B. diandrus* allows this invasive grass to establish even in the presence of morphologically similar native species with greater relative growth rates. Second, positive plant-soil feedback in *B. diandrus* attributed to the fine arbuscular mycorrhizal fungi contributes to its overall success. Lastly, strong soil legacies in abandoned agriculture also contribute to *B. diandrus* invasion and inhibit successful reestablishment of native plants. Root fungal pathogens found in abandoned agricultural fields result in decreased biomass of some native species as well as *B. diandrus*. A greater understanding of the mechanisms contributing to *B diandrus* invasion success suggests that restoration attempts should seed with functionally similar natives while manipulating germination cues and utilize facilitated microbial inoculations to reduce *Bromus diandrus* establishment. However, many mechanisms contribute to the overall success of this invasive species making it competitively superior, and eradication of *B. diandrus* on a large scale is unlikely.

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INTRODUCTION

Anthropogenic disturbances have resulted in changes to the structure and function of ecosystems worldwide (Vitousek et al. 1997). Human induced land use change is a driving force in the loss of biodiversity (Vitousek et al. 1997) and establishment of invasive species (Hobbs 2000). Agricultural practices modify an entire landscape and alter the plant community (Ojima et al. 1994, Vitousek et al. 1997) both upon establishment and their subsequent abandonment. Ecological, social, and economic factors combined have led to increases in abandoned agricultural and farmland systems globally (Ramankutty and Foley 1999). In the United States alone there are an estimated 68 million hectares of abandoned agricultural fields (Zumkehr and Campbell 2013). This abandonment has led to some of the most dramatic plant invasions (MacDonald et al. 2000).

Biological invasions pose serious ecological and economical threats. When invasive species establish and spread, changes to ecosystem structure and function result (Vitousek et al. 1996). Invasive species have strong impacts on nutrient cycling (Vitousek 1990, Ehrenfeld 2003, Vilà et al. 2011), fire regimes (D'Antonio and Vitousek 1992), and primary productivity (Vilà et al. 2011). They are the second leading threat to biodiversity (Primack 2000), and a leading cause of recent species extinctions (Gurevitch and Padilla 2004). The damages associated with invasive species, including environmental impacts combined with the cost of eradicating invasive species, has been estimated at $120 billion/year in the US (Pimentel et al. 2005). Globally, these costs are estimated at $1.4

trillion dollars, nearly a fifth of the global economy (Pimentel 2002). Restoration of invaded old fields will play a critical role in our efforts to conserve both biodiversity and native ecosystem functions.

Invaded plant communities develop from potential species pools through ecological assembly rules (Diamond 1975, Keddy and Weiher 1999). Diamond (1975) coined the term 'assembly rules' to denote a set of rules that determine community structure and development. His rules assert that interspecific interactions, specifically competition, lead to nonrandom co-occurring patterns. Diamonds' use of descriptive data and the assumed mechanism of competition to generate assembly rules was widely criticized (Connor & Simberloff 1979, Keddy 1992). Nevertheless, explanations of species co-existence and invasion have largely relied on intrinsic differences in the competitive ability of species (Chesson 2000, Tilman et al. 2006).

Ecological filters act on regional species pools to determine local plant community assembly (Funk et al. 2008). Ecological filters can be considered geographical, environmental, or biological barriers that work cumulatively and prevent some species from establishing in a local community (Keddy 1992, Funk et al. 2008). Over the past decade many potential mechanisms contributing to an invasive species ability to overcome ecological filters have been proposed and tested. These mechanisms include but are not limited to the evolution of competitive ability (Ellstrand and Schierenbeck 2000), enemy release (Keane and Crawley 2002), novel weapons (Callaway and Ridenour 2004), invasive species traits (Rejmánek and Richardson 1996), niche differentiation and resource-use efficiency (Fridley et al. 2007, Funk and Vitousek

2007), propagule pressure (Simberloff 2009), plant-soil feedbacks (Klironomos 2002), and enhanced mutualisms (Vogelsang and Bever 2009). These theories of invasion are not mutually exclusive, and understanding biological invasions will rely on our ability to integrate them (Shea and Chesson 2002).

I consider three potential mechanisms contributing to the success of a Mediterranean annual grass, *Bromus diandrus*, in abandoned citrus agricultural lands (Fig 1.1). Plant functional traits, plant-soil feedback, and soil legacy effects may all contribute to the success of *B. diandrus*, and understanding how these mechanisms affect plant community composition is necessary for successful restoration.

Plant Functional Traits and Invasion

Elton (1958) suggests that diverse plant communities would be less susceptible to invasion due to differences in resource acquisition strategies among species that leads to less available resources. Since then, the idea that increased biodiversity begets stability in plant communities has been supported by numerous studies (Tilman et al. 2001, Tilman et al. 2006). Others suggest that it is not an increase in species richness, but rather an increase in the functional diversity, or the value and range of species traits, within a community that results in biotic resistance to invasion (Dìaz and Cabido 2001, Pokorny et al. 2005). Functional traits are considered to be adaptations of species to their environments and, therefore, can reveal ecological niche differentiation.

Through limiting similarity (MacArthur and Levins 1967), functional traits of coexisting species are more dissimilar due to the avoidance of competitive exclusion

through niche differentiation (Keddy and Weiher 1999, Funk et al. 2008, Gotzenberger et al. 2012). Therefore, invasive species with functional traits similar to the native species present in their novel environment may be less likely to establish. Studies have demonstrated that functionally similar recipient communities reduce invader colonization, thus supporting the theory of limiting similarity (Emery 2007, Price and Pärtel 2013). Successful restoration of invaded plant communities should therefore attempt to select native species that are similar to the problematic invader in their resource-use characteristics (Funk et al. 2008). However, monocot invasive species have not been affected by within-functional group competition (Cahill et al 2008, Price and Pärtel 2013), and it is unknown if restoration with functionally similar native forb species can reduce monocot invader colonization. Invasive species may still invade functionally similar resident communities when fitness inequalities between native and invasive species results in a competitively superior invader.

Plant-soil Feedback Framework

Over the last decade, theoretical and restoration ecologists alike have emphasized the importance of below-ground interactions in community assembly and invasive species proliferation (Allen and Allen 1984, Klironomos 2002, Ehrenfeld 2003, Callaway et al. 2004, Ehrenfeld et al. 2005). Invasion theories that assume competition and resource partitioning as major forces in plant community assembly may have limited success in explaining community demographics because they ignore the soil microorganisms (Bever et al. 2012). Recent studies have demonstrated that the soil

microbial community acts as a driver of the plant community (Grime et al. 1987, van der Heijden et al. 1998, Klironomos 2002, Ehrenfeld 2003, Callaway et al. 2004, Ehrenfeld et al. 2005, Bever et al. 2012), and therefore incorporating soil microbes into plant community dynamics is necessary in order to gain an understanding of community assembly.

The plant-soil feedback framework incorporates the soil microbial community and is readily related to other coexistence theories (Chesson 2000, Bever et al. 2012). Plant-soil feedback is defined as changes in plant species composition that result in changes in soil conditions, which in turn cause further changes in the plant community, and vice versa (Bever et al. 1997). Positive plant-soil feedback is generally associated with changes in the density of host-specific mutualists, whereas negative plant-soil feedback results from an accumulation of host-specific bacterial and fungal pathogens (Bever et al. 2012).

Both mycorrhizal fungi and pathogenic fungi contribute to plant community composition. Arbuscular mycorrhizal fungi (AMF) significantly influence plant diversity (Grime et al. 1987, van der Heijden et al. 1998, Hartnett et al. 1993, Scheublin et al. 2007), the success of plant invasion (Hawkes et al. 2006, Stampe and Daehler 2003, Pringle et al. 2009), and the success or failure of restoration in abandoned agriculture (Richter and Stutz 2002, Bever et al. 2003, Middleton and Bever 2012). In the Midwestern United States, restoration of native prairie in abandoned agriculture with low mycorrhizal potential has had improved success using mutualist facilitation (Miller 2002, Richter and Stutz 2002, Bever et al. 2003, Middleton and Bever 2012). Invasive species

may create a positive plant-soil feedback in their new environment through enhanced mutualisms with AMF (Vogelsang and Bever 2009).

Local accumulation of pathogens is a common cause of negative feedback in nature and has been suggested as a mechanism for maintaining plant diversity (Mills and Bever 1998, Petermann et al. 2008, Bever 2012). Many native plant species experience negative feedback due to the build-up of host specific fungal pathogens (Kulmatiski et al. 2008, Klironomos 2002). Invasive species may experience positive feedback due to differences in microbial communities between native and adoptive ranges, or the release of natural enemies (Keane and Crawley 2002, Agrawal et al. 2005). Many studies focused on plant-soil feedbacks treat soils as a "black box" and make no attempt to distinguish the microorganisms involved. As a result the role and identity of the soil microorganisms in plant species composition is the least understood mechanism contributing to plant community composition (De Deyn and Van der Putten 2005, Maron et al. 2011). This is due in part to difficulty in identifying soil microbes prior to advances in soil metagenomics, and high variance in the soil microbial community in time and space.

Soil Legacy Effects

Few studies have focused on how land-use history impacts the soil microbial community, plant growth, and competition (Kulmatiski et al. 2006). Soil legacies are the physical, chemical, and biological attributes that remain belowground following change to an ecosystem. Legacy effects on abiotic and biotic properties of soils can result in

priority effects that affect plant community assembly (Kardol et al. 2007, van der Voorde et al. 2011). Legacy effects have been demonstrated to outweigh short-term effects of plants-soil feedback (Kulmatiski and Beard 2008, Elgersma et al. 2011), and studies have demonstrated that the overall soil microbial community is insensitive to short term changes to the plant community (Marshall et al. 2011).

Highly disturbed lands, such as ex-arable land, have been shown to contain low mycorrhizal inoculum potential (Allen and Allen 1980) while containing aggressive soil-borne pathogens (Mills and Bever 1998). Strong soil legacies in abandoned agriculture might inhibit successful reestablishment of native plant species (Cramer et al. 2008) and contribute to invasive species success. Studies have demonstrated that cultivation leaves behind soil abiotic constraints on the re-establishment of native plant communities (Cramer et al. 2008), but little is known about how soil-borne pathogen legacies affect the reestablishment of native plant species and the success or failure of restoration in these systems. Distinguishing the relative importance of soil legacy constraints on restoration of abandoned agriculture can be confounded by the dominance and persistence of invasive plant species and their plant-soil feedback effects (Kulmatiski and Beard 2008, Morris et al. 2013). Recently abandoned citrus agriculture coupled with years of severe drought in southern California provides a community where citrus soil legacy effects can be examined separately from plant-soil feedback effects. This research is the first to identify crop fungal and oomycete pathogens that have a legacy effect on native plant establishment.

Study Site

The research was conducted in CSS vegetation that was converted to citrus agriculture. California's CSS communities extend from as far north as San Francisco to as far south as Rosario, Baja California (Kirkpatrick and Hutchinson 1977). Natural and anthropogenic disturbances have resulted in a loss of 70-90% of CSS in California (Barbour et al. 2007), and unknown losses of associated native forblands that were once likely extensive throughout areas mapped as CSS (Minnich 2008). This significant loss has ultimately caused 11 mammals, 26 birds, and 10 reptiles to be listed as threatened or endangered (Barbour et al. 2007). In addition, California's coastal sage scrub vegetation supports approximately 200 sensitive plant species (Barbour et al. 2007). Most of California's remaining coastal sage scrub has been widely invaded and consequently converted to exotic annual grasslands with *Bromus diandrus* being one of the most abundant species (Barbour et al. 2007).

The research site was at the Lake Mathews-Estelle Mountain Reserve in Riverside County which includes 20, 200 ha of CSS that was converted to citrus agriculture and subsequently abandoned. Citrus orchards have been removed, and attempts to restore CSS are underway as proscribed by the Multispecies Habitat Conservation Plan to comply with the Endangered Species Act. However, the land has been heavily invaded by the exotic annual grass *Bromus diandrus*, and has transitioned to exotic annual grassland. Exotic grasses provide poor quality habitat for native species and of particular concern is the Stephen's kangaroo rat, *Dipodomys stephensi* (Goldingay & Price 1997). The Stephen's kangaroo rat is a federally listed endangered species endemic to Riverside and

San Bernardino Counties, and its natural habitat is sparse grassland dominated by forbs (Price *et al.* 1994). The Lake Mathews-Estelle Mountain reserve was established as Stephen's kangaroo rat habitat. Control of exotic grasses followed by the establishment of native forb species is needed.

Conclusions

My dissertation research broadly considers mechanisms underlying plant community assembly and plant invasions (Fig 1.1). The three main objectives of my dissertation were to 1) examine biotic resistance of a native plant community through limiting similarity, 2) determine how AMF and non-mycorrhizal fungi contribute to plant community assembly through plant-soil feedback, and 3) determine how soil biotic legacies affect the growth and establishment of native and invasive species during the restoration of invaded plant communities. Through both large-scale field experiments and targeted greenhouse experiments, I manipulated competition and the soil biota to gain a better understanding of these three mechanisms in the success of *Bromus diandrus* invasion.

Understanding the relative importance of mechanisms underlying community assembly is a fundamental question in ecology. Plant invasions are "natural experiments" that provide ecologists with many opportunities to study community assembly and successional processes (Cramer and Hobbs 2007). Furthermore, restoration of these invaded lands has the potential to influence community assembly patterns by manipulating environmental filters, and provides insight to those important mechanisms

determining local plant community composition (Funk et al. 2008). Control of invasive plant species will rely on an understanding of the direct competitive effects of invasive species, as well as the indirect effects on soil biota that may facilitate invasion while also inhibiting the re-establishment of natives. Here, the recent invasion of an abandoned citrus agricultural area provides a unique system in which I study both above and below ground environmental filters of plant communities through restoration. This important research can then be used to inform successful restoration of CSS.

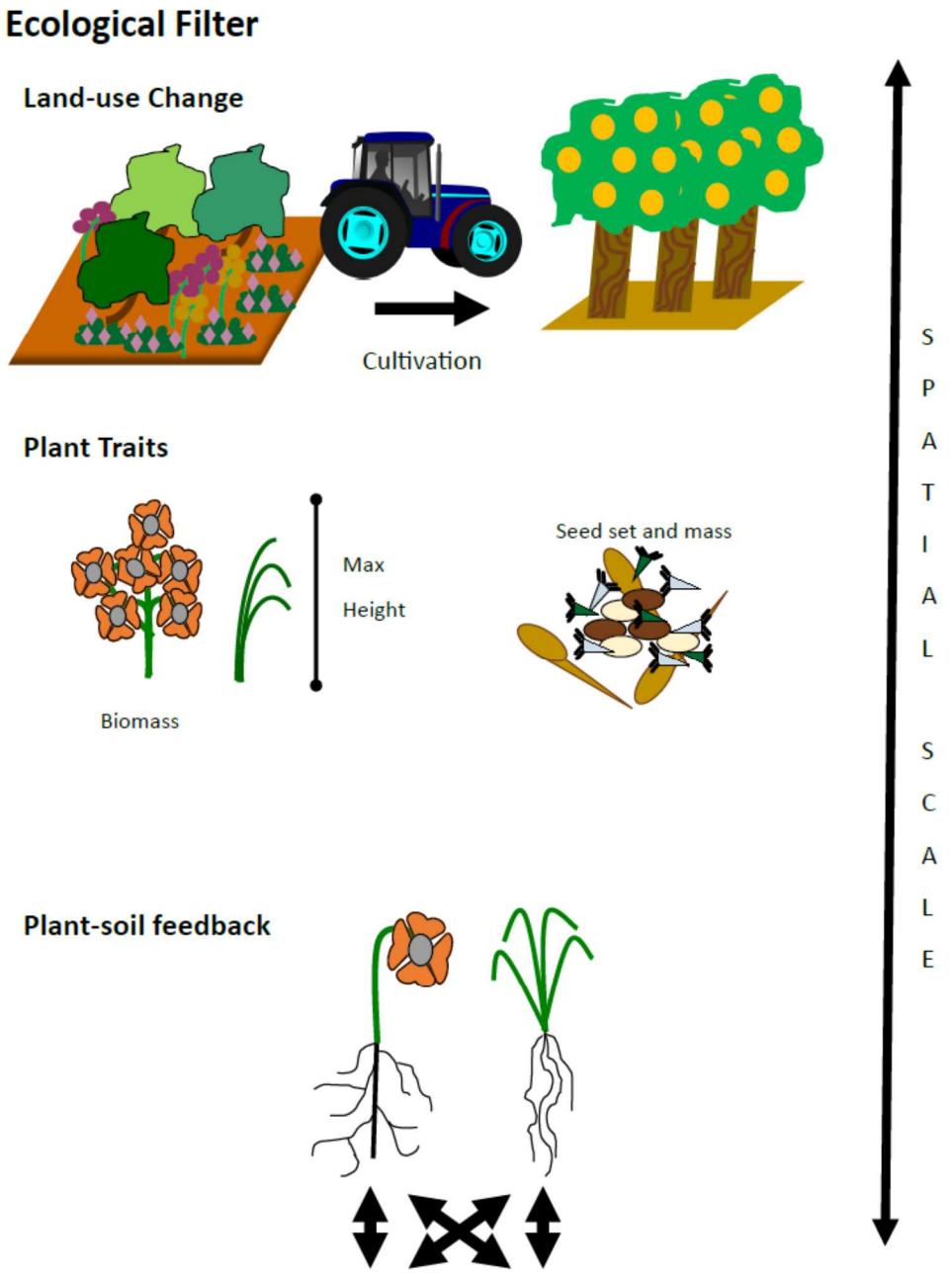

Figure 1.1: Ecological filters act at multiple scales to determine local community composition from a species pool. Cultivation converts native vegetation to citrus groves, causing plant invasions and leaving large-scale soil legacy effects. Plant traits such as height, seed set, or seed mass may increase competitive ability of native with invasive species. Native and invasive plants experience feedbacks with their own or their neighbor's soil microorganisms as shown by arrows (illustration by B. Hilbig).

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RESTORATION OF INVASIVE ANNUAL GRASSLANDS USING PHENOLOGY AND MORPHOLOGICAL TRAITS

ABSTRACT

Limiting similarity theory suggests that ecological communities are susceptible to invasion when invasive species are functionally different from native species. Therefore, restoring invaded plant communities using native species with similar traits to the problematic invasive species might result in greater biotic resistance. I experimentally examine how phenology, plant morphological traits, and root-associated fungi define vegetation structure and development during restoration of an invaded annual grassland in southern California dominated by exotic *Bromus diandrus* and *Erodium cicutarium*. Phenology and morphological traits were used to define four plant functional groups each represented by two locally abundant species 1) tall winter annual forbs, 2) short winter annual forbs, 3) perennial winter forbs and 4) summer annual forbs. Functional groups were seeded with and without the two invasive species, and vegetation data were collected to assess biotic resistance to invasion by these species. While many studies have demonstrated successful exclusion of exotic species through the establishment of functionally similar native species, I found no evidence for limiting similarity. Seeding with functionally similar native forbs did not significantly affect the establishment and growth of *Bromus diandrus* or *Erodium cicutarium* in our study. While the native forb species chosen in this restoration study were similar in measured morphological traits, they varied in germination time, with invasive species germinating earlier than native

species. This critical difference in phenology might result in stabilized niche differences that give invasive species a competitive advantage and trumps any effects of limiting similarity in morphological traits. Lastly, increases in native summer annual thatch were positively correlated with increases in native winter annual forb cover in the subsequent growing season, but there was no correlation with invasive species density or cover. Including native summer annuals may promote restoration of native winter annuals. However, restoration using limiting similarity theory that relies on morphological similarities and ignores differences in phenology may be ineffective in increasing biotic resistance to invasion in the resident community.

INTRODUCTION

Invasive species pose a serious threat to the functioning and stability of an ecosystem. Ecological restoration of invasion-resistant plant communities will play a key role in our efforts to maintain biodiversity, productivity, and ecosystem functioning (Funk *et al*. 2008). Biotic resistance of a community to invasion occurs when species within the community limit the establishment of invasive species from a regional species pool (Levine et al. 2004, Byun et al. 2013). Explanations for biotic resistance often focus on resource competition and trait differences between the invader and resident species (Funk et al. 2008, Drenovsky et al. 2012, Godoy and Levine 2014). Understanding which species traits present in a community lead to biotic resistance can guide restoration of invaded systems.

Limiting similarity theory (MacArthur and Levins 1967) predicts that community assembly is not random and, through niche differentiation, coexisting species will exhibit different resource-use traits as to avoid competitive exclusion (Keddy and Weiher 1999, Chesson 2000, Funk et al. 2008, Gotzenberger *et al*. 2012, Price and Pärtel 2013). Invasive species are considered less likely to establish if native species with similar resource-use traits are present (Fargione et al. 2003, Ordonez et al. 2010). Restoration of invaded systems should therefore utilize native species that are similar to the problematic invader in their resource-use traits (Funk *et al*. 2008, Cleland et al. 2013). Evidence supporting limiting similarity for forb invaders has been demonstrated (Price and Pärtel 2013), whereas monocots have been unaffected by within-functional group competition (Cahill *et al*. 2008, Price and Pärtel 2013).

Functional groups of plant species can be defined informally or based on mathematical algorithms. They have been defined using flowering phenology as a functional trait (Craine *et al*. 2012), resource pools utilized and resource use efficiency (Fargoine 2003), or leaf traits (Ackerly and Reich 1999). Traditionally used functional groups defined by life form include C_3 and C_4 grasses, forbs, shrubs, and trees. However, these traditional pre-described functional groups do not always adequately describe traits that are important to biotic resistance (Byun et al. 2013) nor do they describe similarities in phenology. Phenology may regulate competitive interactions between invasive and native species, as invasive species often exploit temporal niches not occupied by other species (Wolkovich and Cleland 2010, Cleland et al. 2013, and Byun et al. 2013, Godoy and Levine 2014). Additionally, traditional functional groups may consider nitrogen

fixers but seldom consider differences in plant species dependence and responsiveness to mycorrhizal fungi. Plants can obtain up to 80% of their nitrogen requirement, and up to 90% of their phosphorus requirement through arbuscular mycorrhizal symbionts in a natural ecosystem (van der Heijden and Horton 2009). Many ruderal invasive species are considered non-mycorrhizal (Pringle et al. 2009), while native species may rely on the mutualism for resource acquisition. Variance in dependence on mycorrhizal fungi between species has direct consequences for competition and plant community assembly (Allen and Allen 1984, Hawkes et al. 2006, Callaway et al. 2007, Seifert et al. 2009, Hilbig and Allen 2015).

Ruderal invasive annual grasses and forbs have invaded most coastal sage scrub (CSS) communities in southern California (Cox and Allen 2008a, Kimball et al. 2014). Invasion of CSS by *Bromus diandrus,* a dominant Mediterranean annual grass, results in altered fire frequencies (D'Antonio and Vitousek 1992, Brooks et al. 2004), shifts in soil resources (Dickens et al. 2013), and ultimately vegetation type conversion to invasive annual grassland (Barbour et al. 2007, Minnich 2008). Complete removal of invasive grasses may lead to increases in ruderal invasive forbs (Cox and Allen 2008b, Allington et al. 2013), such as *Erodium cicutarium*. Because this is a common phenomenon in invaded systems, limiting similarity studies that use a single invasive species to make conclusions about community invasibility should be interpreted with caution (Emery 2007). Restoration of CSS invaded with invasive grasses should consider subsequent forb invasion.

Little is known about the role of summer annuals in the establishment of native and invasive species in CSS, and the use of summer annuals to restore southern California CSS and forbland has never been reported. Summer annuals typically senesce before fall precipitation initiates growth of winter annuals, so the main interaction would be via remaining thatch rather than a direct competitive effect. Thatch consists of the previous season's plant debris (Cox and Allen 2008b). A native community containing summer annuals may limit or promote the establishment of invasive or native species by altering microsite availability with summer annual forb thatch. Removal of winter annual thatch has been shown to reduce exotic grass (Cox and Allen 2008b) and forb establishment (Matzec and Hill 2012), but it remains unknown how native summer annual thatch affects establishment. In this study I use both winter annual native forb species differing in phenology and morphological traits and summer annual forbs in restoration of invaded CSS dominated by *Bromus diandrus* and *Erodium cicutarium*. I hypothesized that 1) seeding with native forbs that are functionally and morphologically similar to *B. diandrus* and *E. cicutarium* would result in greater exclusion of the invasive grass and forb respectively, and 2) native summer annual thatch will affect both native and invasive seedling establishment.

MATERIALS AND METHODS

Study Site

This study was completed within Lake Mathews Riverside County Habitat Conservation Agency (RCHCA) land located in southwestern Riverside, CA (33.36°N, 117.02°W, elevation: 450 m). This Mediterranean-type climate has a mean annual rainfall of 250 mm, most of which occurs in the winter growing season. Precipitation during the years of study was 118 mm in 2012-2013, 140 mm in 2013-2014, and 173 mm in 2014-2015 (July 1-June 30). RCHCA lands once had extensive stands of coastal sage scrub (CSS) with an open canopy of shrubs such as *Artemisia californica* and *Eriogonum fasciculatum*, and interspaces filled with native annual forbs (Minnich 2008). These lands were converted to citrus agriculture and abandoned approximately 20 years ago. Current vegetation is dominated by the invasive annual grass *Bromus diandrus* and the invasive annual forb *Erodium cicutarium* with few remnant CSS shrubland areas. The area is undergoing endangered species habitat restoration for the Stephen's kangaroo rat.

Experimental Design

In the fall of 2012, I established eighty-five 1 x 1m experimental plots in five replicate blocks with 17 treatments. Vegetation was removed from all but control plots with glyphosate applied at manufacturer's lowest recommended rate in October 2012 and then raked to remove thatch. Three weeks after herbicide treatments bare plots were sowed with seeds of predefined forb functional groups. Phenology and morphological

traits were used to define four plant functional groups: 1) perennial forbs, 2) tall winter annual forbs, 3) short winter annual forbs, and 4) summer annual forbs. Each functional group was represented by two of the most locally abundant native species, chosen based on a 2010 vegetation assessment (Allen *unpublished data*). Perennial winter forbs included *Heterotheca villosa* and *Lessingia filaginifolia*. Tall winter annual native forbs included *Amsinckia menziesii* and *Layia platyglossa*. Short winter annual native forbs included *Plantago erecta* and *Lasthenia californica*. Summer annual forbs included *Hemizonia fasciculata* and *Croton setigerus* (nomenclature from Jepson 2013). Prior to sowing seeds, the germination rate of all species was determined and used to calculate a total seed application rate of 200 viable seeds/m^2 per species. Plots of 1 x 1 m received seeds of one of 17 treatments (Table 1.1): each winter forb functional group alone, all winter forb functional groups together, each functional group with invasive species *B. diandrus* and *E. cicutarium,* all winter forb functional groups together with invasive species, each functional group with summer annuals, all winter forb functional groups together with summer annuals, each functional group with invasive species and summer annuals, all winter forb functional groups together with invasive species and summer annuals, or left as an unseeded control. The treatments were randomly assigned within each of 5 replicate blocks. I used an additive design so that intraspecific competition in native species was the same between plots with and without invasive species (Kimball et al. 2014). Lake Mathews experienced severe drought in both the 2012-2013 and 2013-2014 growing seasons. However, irrigation was available in time for the 2013-2014 growing season. Plots were reseeded in November 2013 and were irrigated with

approximately 175 mm (~35 mm weekly from Jan 1, 2014 to February 7, 2014) using an overhead sprinkler with a municipal water source. Perennial forbs failed to germinate and establish in the field, and those plots were eliminated from analyses. Despite its presence at the field site and high germination in the lab, *C. setigerus* failed to germinate in the field plots. Eight invasive species including *Salsola tragus*, *Hirschfeldia incana*, *Brassica nigra*, *Chenopodium alba*, *Lactuca serriola, Malva parviflora*, *Sisymbrium irio*, and *Schismus barbatus* germinated from the seedbank. These species seedlings were recorded and subsequently removed from all plots except controls because the goal of the experiment was to test interactions between functionally similar native and invasive plants. Seedlings were removed when still young and with care to minimize soil disturbance. All plots were monitored through the 2013-2014 growing season, and in addition plots containing summer annual forbs were monitored through 2014-2015 but not irrigated. The last date of monitoring was January 2015, as severe drought caused major mortality and the termination of the study.

Trait Measurements

Relevant plant phenology and morphological traits were measured for each winter annual species on a representative sample of ten individuals from plots containing all functional groups and invasive species across all five blocks. Plant height and biomass were measured on individuals at peak flowering time. Potential seed set for each species was estimated in April 2014 by counting flowers per individual as a proxy for seed set as described by Kimball et al. (2014). Seed mass was determined by weighing 10 harvested

seeds from each of 10 individuals of each species. Due to the difficulty in differentiating between germination and emergence in the field, timing of germination after first precipitation or irrigation event was observed both in the greenhouse and field. In the greenhouse, I used 25 seeds of each species potted in field-collected soils and monitored when seedlings emerged following watering to field capacity. In the field I observed seedling emergence following the first rain event.

Root colonization by arbuscular mycorrhizal fungi (AMF) and ascomycete fungi was measured for all winter annual species. Fungi of the Ascomycota range from purely saprophytic to obligate pathogens, and include important plant pathogens (Webster and Weber 2007). Ten individuals of each species were destructively harvested for their fine roots using a trowel to 10 cm deep and keeping the stem with attached roots intact for correct species identification. To assess fungal colonization, roots 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). Percent colonization of fine AMF, coarse AMF and ascomycetes was estimated using a modified magnified intersection method as described in Hilbig and Allen (2015).

Sampling Effort

Plots were sampled at peak flowering time for plant density, percent cover, and plant biomass in March 2013 and 2014. Plant density was counted within gridded 0.5m x 0.5m quadrat frames centered in plots, and percent cover was estimated to the nearest 1% in the same quadrats. Plant biomass per quadrat was clipped at 1%, 5%, 11%, 25%, 50%,

75%, and 100% cover within gridded frames in the treatment plots seeded with all functional group and invasive species (treatment 14, Table 1.1). Biomass was dried at 60°C for 48 hours and then measured. Regression analyses of clipped biomass vs cover value were performed to calculate biomass based on cover values per functional group. Relative growth rate (RGR) was calculated for the mean relative growth rate of all species as:

$$RGR = (log_e W_2 - log_e W_1)/(t_2 - t_1) \quad (1)$$

Where t is time and W is dry weight (Hunt et al. 2002), and W_1 is seed mass. Ten individuals per species growing within plots seeded with all functional groups were destructively harvest in April 2014 and used to calculate RGR.

Plots containing summer annuals were sampled at peak flowering time for plant density, percent cover, and plant biomass in March and May 2014, and for seedling density and cover in Jan 2015. Plant density, cover and biomass were estimated as described above. Percent cover of summer annual thatch was estimated in October 2014 as attached and flattened stems of *H. fasciculata* that have remained in the plot from the 2013-2014 growing season. Exceptional drought in the 2014-2015 growing season resulted in high seedling mortality by March 2015, and sampling at peak flowering was not feasible.

Statistical Analysis

Due to low establishment in the 2012-2013 growing season, only traits and community composition measured in the 2013-2014 growing season were analyzed and

reported. Prior to analyses, plant species were grouped into functional groups based on phenology, morphology, and native/ invasive status (Prevéy et al. 2014). Each morphological trait and relative growth rate was analyzed by separate one-way ANOVA with species as a fixed factor, followed by least significant difference ($L.S.D._{0.05}$). Individuals used to measure traits were average sized (Kimball et al. 2014) to exclude outliers. Percent root colonization was analyzed using separate one-way ANOVA for coarse AMF, fine AMF, and ascomycete fungi with species as a fixed factor. Plant density, biomass, and percent cover for native and invasive species were analyzed using separate one-way ANOVA for each species and for native and invasive species grouped, with functional groups as a fixed factor. Functional groups were compared using $L.S.D._{0.05}$. To test the effect of summer annual thatch on native and invasive species establishment, January 2015 seedling density and cover data were regressed against the percent cover of *H. fasciculata* thatch in plots with and without summer annuals. All data were checked for homogeneity of variances using Levene's tests, and for normality using the Shapiro-Wilk test. Non-normal data were natural log transformed when appropriate to meet the assumptions of ANOVA. Box and whisker plots and Mahalanobis distance were used to detect outliers, which were removed prior to statistical analyses. All statistical analyses were performed using R Studio version 3.0.2 (R Development Core Team 2013). Significance was determined at $\alpha=0.05$.

RESULTS

Traits

Plant height varied significantly across all species ($P<0.001$, Fig 2.1a), and post-hoc analysis showed that *B. diandrus* and *A. menziesii* height was greatest and did not significantly differ from one another. Similarly, *E. cicutarium* and *P. erecta* had the lowest plant height and were not significantly different from one another (Fig 2.1a). Aboveground biomass also varied significantly across all species, and like plant height was highest in tall forb species and *B. diandrus* ($P<0.001$; Fig 2.1b). Potential seed set varied significantly across species ($P<0.001$; Fig 2.1c). High seed set was found in both *L. platyglossa* and *L. californica*, whereas invasive species, *A. menziesii,* and *P. erecta*, had low potential seed set. Seed mass varied significantly across species, with *B. diandrus* having the largest seed mass and *A. menziesii* having the second largest seed mass ($P<0.001$; Fig 2.1c). The tall annual forbs *A. menziesii* and *L. platyglossa* had the greatest relative growth rate across species, 0.0712 $gg^{-1}day^{-1}$ and 0.0749 $gg^{-1}day^{-1}$ respectively ($P<0.001$; Fig 2.2). Both invasive species had smaller relative growth rates than all native species except *P. erecta*, with *B. diandrus* having the smallest relative growth rate across all species, 41.1 $mgmg^{-1}mo^{-1}$ ($P<0.001$; Fig 2.2). However, on average *B. diandrus* seeds germinated more quickly after first precipitation than any other species in the greenhouse. By the third day after potting and watering 16 of 25 *B. diandrus* seeds had germinated compared to 9 *L. platyglossa*, 6 *A. menziesii*, 2 *L. californica*, 0 *P. erecta*, and 0 *E. cicutarium*. In the field plots invasive species and *L. platyglossa* germinated immediately following the first significant rain event on November 21, 2013. Seedlings

of *A. menziesiii*, *P. erecta*, and *L. californica* did not emerge in the field until December, almost two weeks behind the emergence of invasive species.

Non-mycorrhizal and mycorrhizal fungi colonized the roots of all native and invasive species (Fig 2.3). Percent colonization by non-mycorrhizal fungi was significantly higher in invasive species and *L. californica* than other native forbs ($P=0.002$; Fig 2.3). Fine AMF colonization was greatest in *L. californica* ($P<0.001$; Fig 2.3). *L. californica* did not significantly differ in its fungal root infection from *B. diandrus.* Coarse AMF infection was greatest in *L. platyglossa, P. erecta,* and *E. cicutarium*, and smallest in *L. californica* and *B. diandrus* ($P<0.001$; Fig 2.3).

Plant community composition

Precipitation events and irrigation resulted in high native plant establishment in the 2013-2014 growing season. The rare native forb *Convolvulus simulans* (listed as sensitive by the California Native Plant Society, http://www.rareplants.cnps.org/), germinated from the seed bank with up to 12 individuals per quadrat establishing, but zero in most quadrats. There was no treatment effect on *C. simulans* establishment, and it was not removed from any plot in which it occurred nor was it destructively harvested. Only two other native species germinated from the seedbank, *Lupinus succulentus* with 5 individuals and *Pectocarya recurvata* with 3 individuals total across all plots.

Native forb density, cover and biomass varied significantly with species and treatment ($P<0.001$ for all variables). In general native forb functional groups had greater establishment in plots where invasive species were excluded. *A. menziesii* had greater

density, cover, and biomass in tall forb plots without invasive species than all other plots (P= 0.044, P=0.005 and P=0.005 respectively; Table 1.2, Fig 2.4). *P. erecta* had greater percent cover and biomass when grown in the short forb functional group plots than with all other functional groups (P=0.001, P=0.002) and greater density in short forb plots than all other plots, except plots with all functional groups present (P=0.001; Table 1.2, Fig 2.4). While *L. platyglossa and L. californica* had the lowest establishment of the native forbs, *L. californica* had greater cover and biomass when grown in the short forb functional group than all other plots (P=0.007, P=0.006). Density, cover, and biomass did not vary significantly in *L. platyglossa* when comparing functional group plots with and without natives.

Invasive plant density, cover, and biomass did not differ significantly among functional group treatments (P>0.05, Table 1.2). All plots in which invasive species were not removed had high percent cover of invasive species (Table 1.2). Control plots that were neither seeded nor weeded had an average of 87% invasive plant cover.

Higher percent cover of summer annual thatch was positively correlated with higher percent cover, but not density, of native winter annual forbs (R^2=0.19, P<0.001; Fig 2.5a). There was no relationship between percent cover of summer annual thatch and invasive species cover or density (Fig 2.5b), but there was a strong negative correlation between native species cover and invasive species cover in January 2015 (R^2= 0.46, P<0.001; Fig 2.6).

DISCUSSION

Applying the theory of limiting similarity to reduce exotic invaders by planting competitive native species had limited success. However, including summer annual forbs increased establishment of native winter annuals, an interaction that has never been examined before. My study demonstrates that summer annual forb thatch resulted in greater native forb cover while having no effect on invasive species. While some studies have shown that invasive species establishment declines with the removal of thatch (Cox and Allen 2008b, Matzec and Hill 2012), others have demonstrated that bare soil can promote the establishment of exotic forb species (Cox and Allen 2011). Invasive species cover was high in plots with 0% cover of summer annual thatch as well as plots with up to 80% cover of summer annual thatch. Differences in the quality and quantity of thatch inputs could explain different results seen in thatch removal studies. The correlation between summer annual thatch and native species cover was significant but weak. Plots were not irrigated beyond the 2013-2014 winter growing season and there was high variation in summer annual establishment and growth as well as winter forb seedling establishment in 2015. Nevertheless, these results suggests that restoration of invaded CSS should include summer annual forbs in seed mixes.

The analysis of fungal functional groups across plant species indicated all three functional groups of fungi were present in all plant species, and small differences in percent colonization by fungal functional groups has no explanatory power in plant biomass and composition in this study. In contrast, other studies have demonstrated that specific functional groups of fungi are related to plant growth and community

composition (Allen and Allen 1984, Klironomos 2002, Callaway et al. 2004, Hilbig and Allen 2015). Percent colonization is measured in a single snapshot of time and then compared to cumulative growth responses of plants. Variation in percent colonization from one study to the next can be due to the timing of destructive sampling and the phenology of fungi at that time (Allen 1991, Smith and Read 2008). Additionally, percent colonization of AMF alone may not be a useful variable in understanding the functioning of mycorrhizae, because it is a calculation that takes into account intercepts of mycorrhizal fungi but not root length (Allen 2001). Large and small root systems may have the same percent infection, but large root systems will have greater infected root length that may affect nutrient uptake and plant growth. These factors may have contributed to high within species and treatment variation in percent colonization that results in poor explanatory power.

Many studies have demonstrated successful exclusion of exotic species through the establishment of functionally similar native species and have promoted restoration by using functionally similar natives (Emery 2007, Cleland et al. 2013). I found no evidence that applying the theory of limiting similarity during restoration results in greater biotic resistance in the native plant community to either invasive species. Seeding with functionally similar native forbs did not significantly affect the establishment and growth of invasive species. The tall forb functional group was best at reducing invasive species, but invasive species cover still exceeded 50% on average in this treatment. A few other studies that applied the theory of limiting similarity to restore invaded plant communities also had limited success (Kimball et al. 2014), especially those studying monocot

invaders (Cahill et al. 2008, Price and Pärtel 2013). Selection of a priori functional traits measured and the native species used might contribute to the success of utilizing limiting similarity theory during restoration. Species that are native regionally may not be locally native, which brings up questions about their appropriateness for restoration. Some studies that have found evidence for limiting similarity create experimental plant communities that contain functionally similar native and invasive species according to a predetermined suite of traits, but contain species that do not naturally occur at the experimental field site without planting or seeding (Cleland et al. 2013). For example, Cleland et al. 2013 used *Aristida adscensionis* as a native annual grass to test limiting similarity in a southern California CSS community invaded by invasive Mediterranean grasses, but *A. adscensionis* is a C_4 grass that does not occur in CSS, but is common in summer rainfall regimes and desert ecosystems (Heske et al. 1993, Valone et al. 2002). While native annual grasses are more similar functionally and morphologically to invasive grasses than the native forbs chosen here, our research site was historically a shrubland and native grass species do not naturally occur here. I therefore did not use regionally native grasses.

The establishment of *P. erecta* and *L. californica* did not significantly reduce the density and cover of functionally similar invasive species *E. cicutarium* compared to all other treatments. *E. cicutarium* has a lower relative growth rate than *L. californica*, but earlier germination in the field combined with high numbers in the seed bank (Cox and Allen 2008a) results in a high number of smaller individuals establishing. Higher relative growth rates in native species than invasive species did not improve their growth and

establishment. This could be due to low number of seeds of native species in the seedbank (Cox and Allen 2008a) despite additional seeding. After the emergence of a seedling, the factor most influential to future growth is the density of seedlings already emerged (Ross and Harper 1972). The sheer number of individuals that establish earlier than native forbs allows *E. cicutarium* to outcompete native forb species.

Molinari and D'Antonio (2014) suggest that species with earlier flowering, larger seed size and greater height will coexist with *B. diandrus*, and these traits should be considered when selecting species to increase native diversity. *B. diandrus* and *A. menziesii* are similar in many measured morphological traits related to the competitive ability of a plant, such as height and biomass, and both flower at the same time of year. Yet, *A. menziesii* was a poor competitor with *B. diandrus* despite *A. menziesii* having a larger relative growth rate. This may be due in part to the difference in the timing of germination after the first rain event. Phenology differences in native and invasive species give an invasive species an advantage when they promote stabilizing niche differences that then make coexistence between these phenologically offset competitors more difficult (Godoy and Levine 2014). Invasive species utilize temporal niches that allow them to establish and gain a foothold in a new system (Godoy et al. 2009). Relative germination date is important in determining relative competitive ability, as offset competition can negatively impact those species germinating later (Boerner and Harris 1991, Orrock and Christopher 2010). Dominance by neighbors can impact early growth stages of an individual, and the growth of an individual is directly related to the emergence rank (Ross and Harper 1972). Other studies have demonstrated that early

phenology in invasive species leads to a competitive advantage (Marushia et al. 2010, Wainwright et al. 2012). The earlier germination and larger seed mass of *Bromus* allows this invasive to establish even in the presence of morphologically similar native species with greater relative growth rates. This suggests that timing of germination or emergence rank, trumps similarities in morphological traits between native and invasive species during annual grass invasion. Studies that consider limiting similarity as a mechanism contributing to community assembly should not only consider resource-use traits but also germination traits that may trump any limiting similarity effects.

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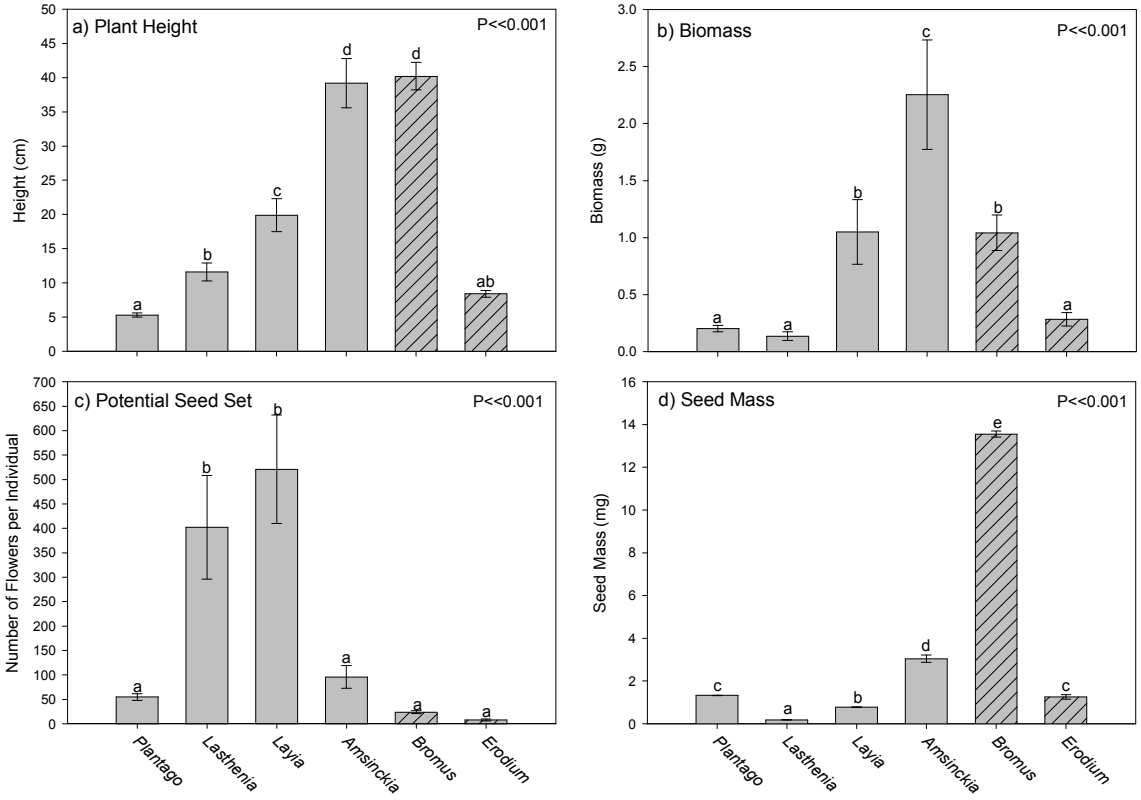


Figure 2.1 - Morphological traits measured on native and invasive species collected from plots that contained all functional groups and invasive species [a) Plant height in cm, b) Potential seeds set based number of flowers per plant, c) Biomass in g/plant, d) Seed mass in g/seed]. Invasive species are displayed with patterned bars. Significance was determined at $\alpha=0.05$.

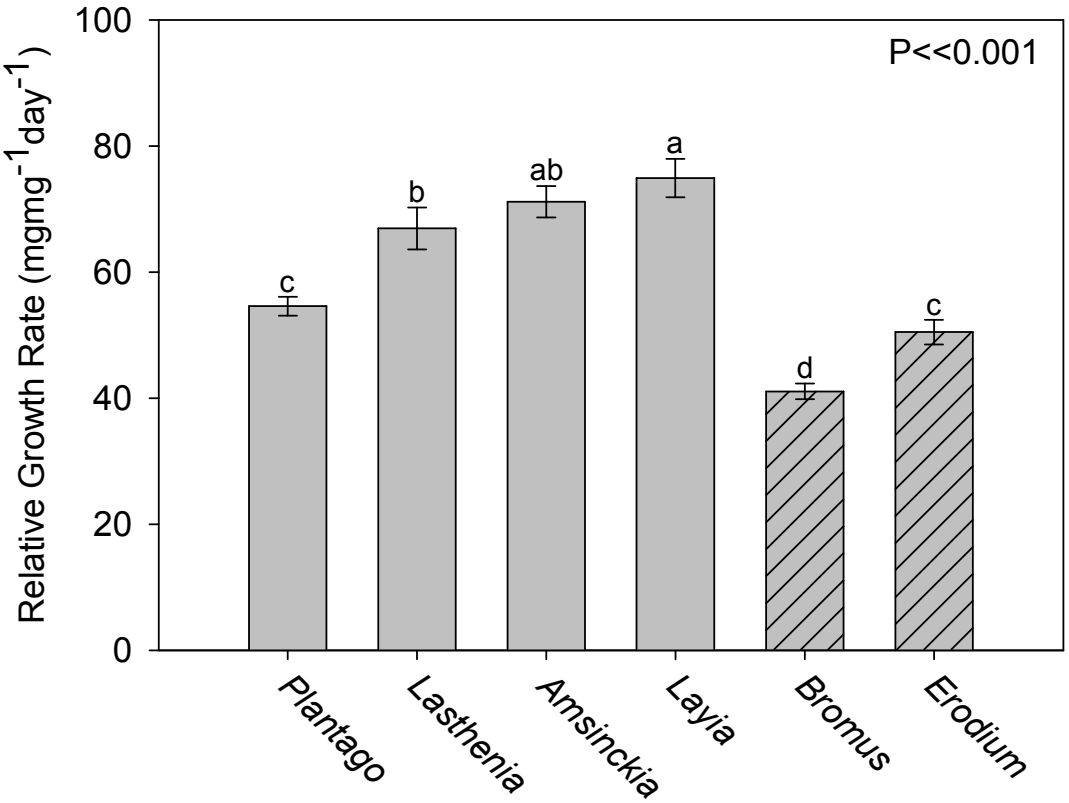


Figure 2.2 - Relative growth rate calculated as $RGR = (log_e W_2 - log_e W_1)/(t_2 - t_1)$ for native forbs and invasive species. Invasive species are displayed with patterned bars. Significance was determined at α=0.05.

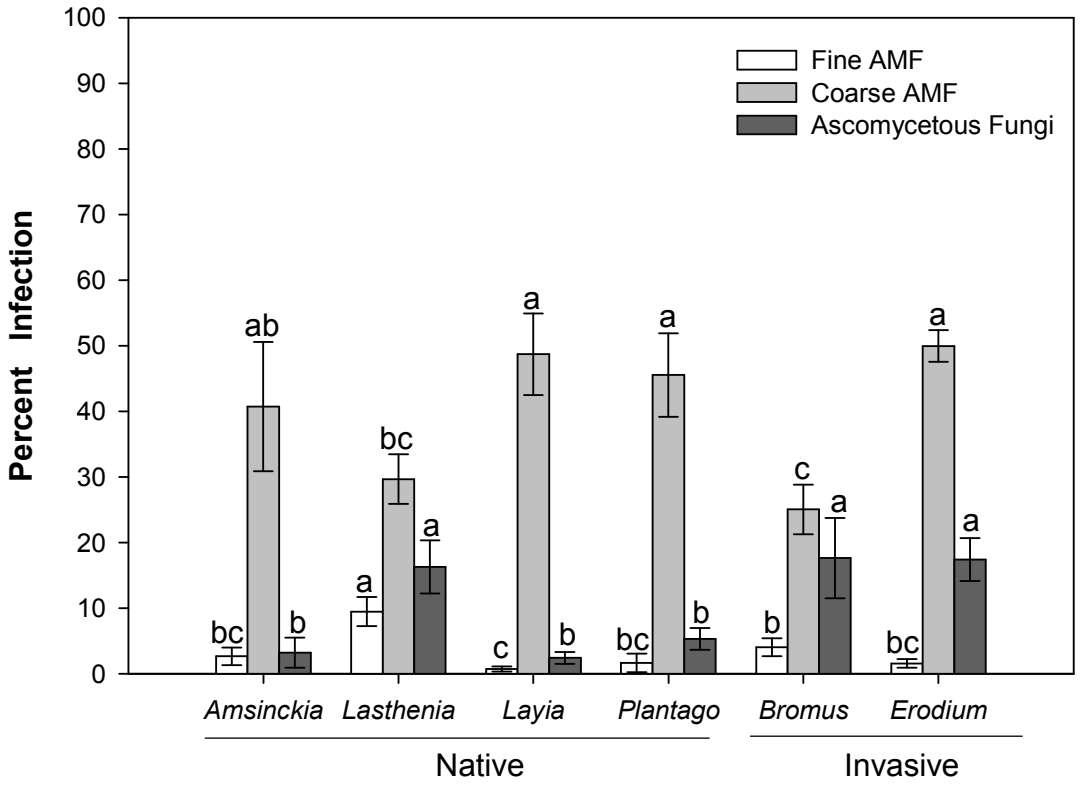
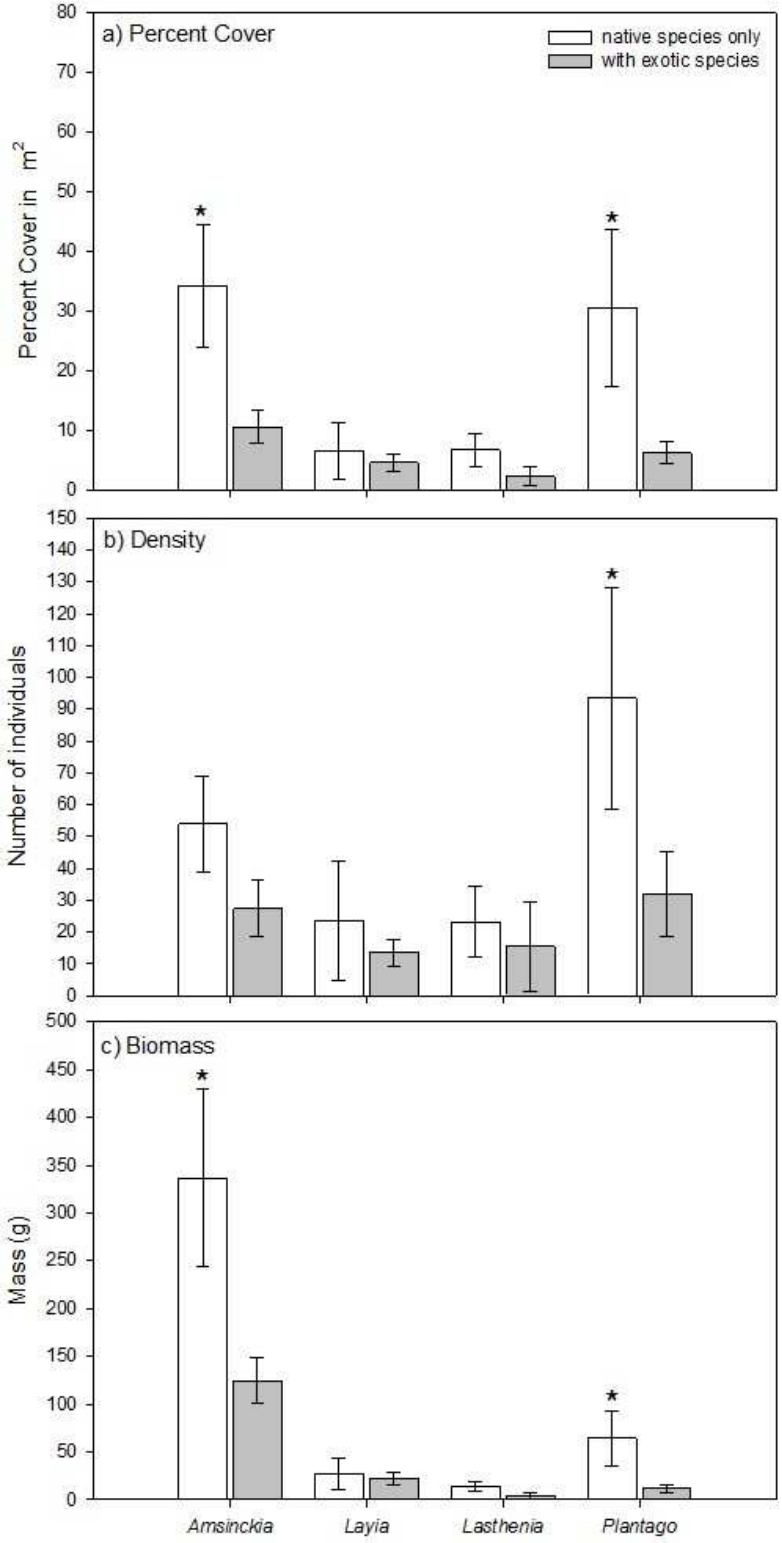


Figure 2.3 - Percent root colonization of fine AMF, coarse AMF and non-mycorrhizal fungi by species. Separate one-way ANOVA for percent colonization with species as a fixed factor was performed for each fungal group. Significance was determined at α=0.05.

Figure 2.4 - Native forb a) density/m2, b) percent cover, and c) biomass/m2 when grown in plots with their functional group without invasive species and their own functional group with invasive species. * shows significance difference in density, cover, and biomass for each species with and without invasive species at α=0.05.



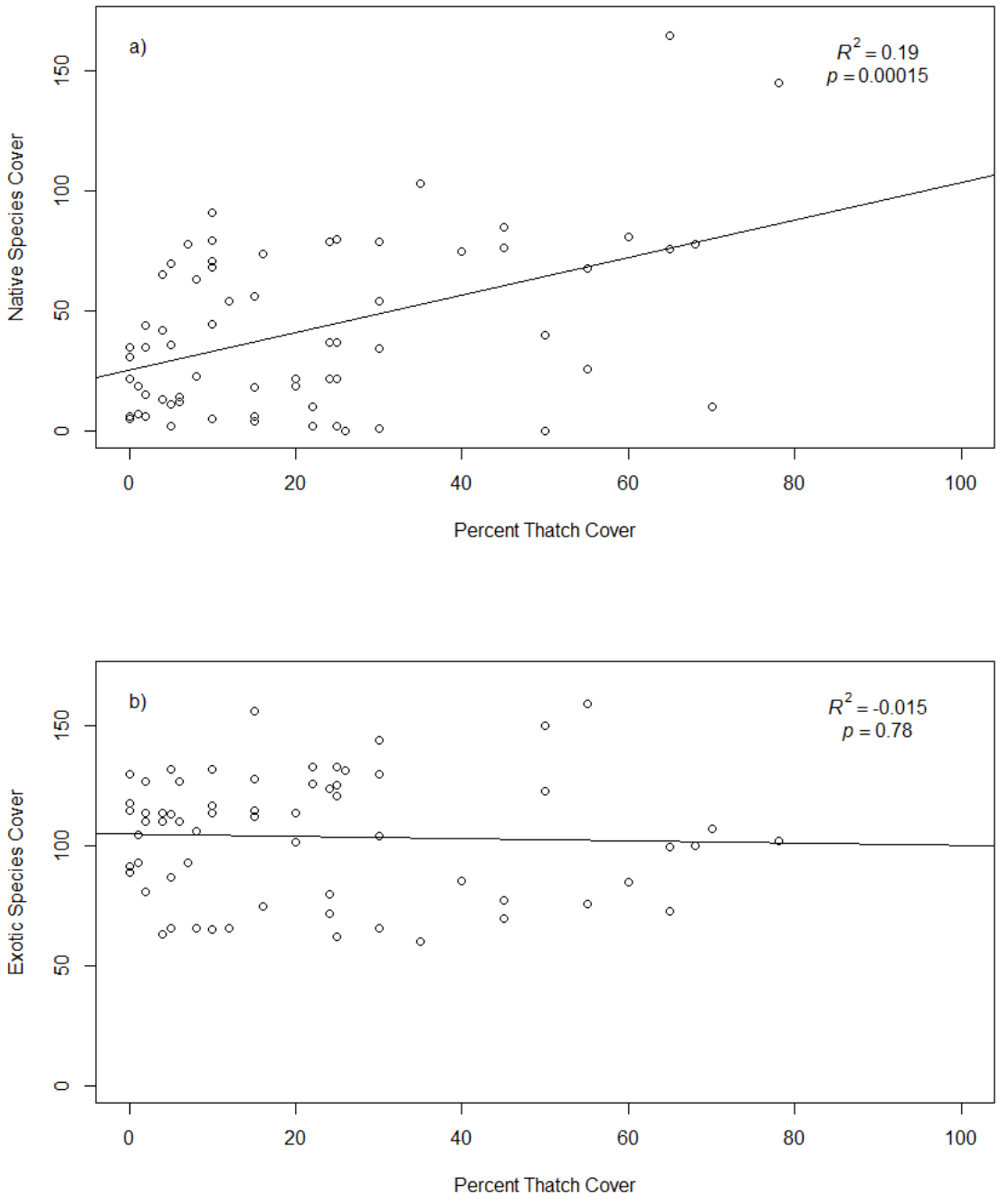


Figure 2.5. Linear regressions comparing a) invasive species cover by percent thatch cover left from summer annuals, and b) native species cover by percent thatch cover from summer annuals in January 2015.

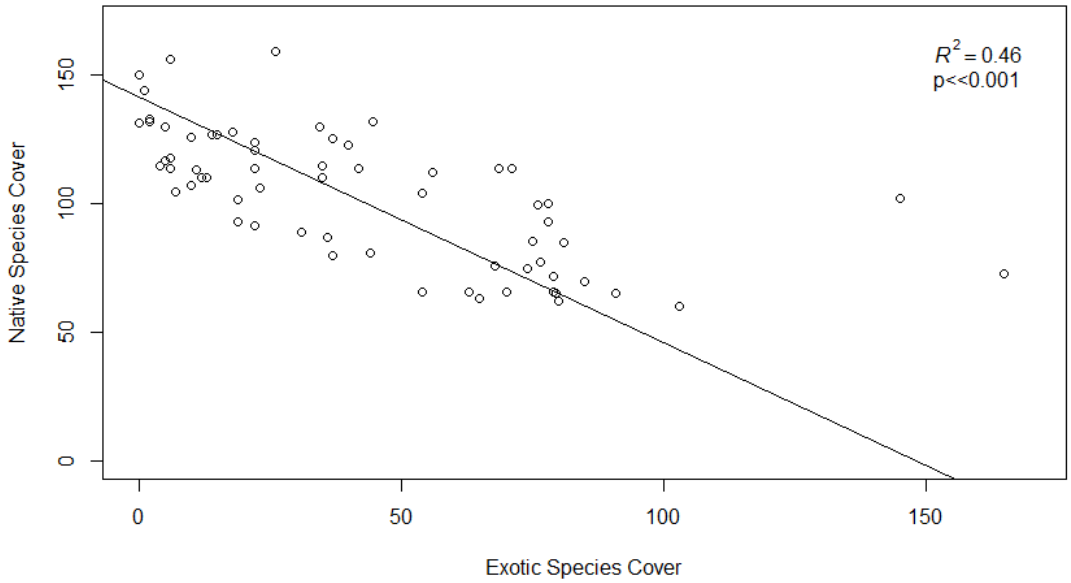


Figure 2.6 - Linear regressions comparing native species cover by invasive species cover in January 2015.

Table 1.1 List of seeding treatments

	Functional Group Seeding Treatments	Species seeded
1)	Control	None
	Plots with Native Forbs Only	
2)	All native functional groups, invasives removed	*A. menziesii*, *L. californica*, *L. platyglossa*, *P. erecta*
3)	Tall winter annual forbs	*A. menziesii* and *L. platyglossa*
4)	Short winter annual forbs	*L. californica* and *P. erecta*
5)	Perennial winter forbs	*H. villosa* and *L. filaginifolia*
6)	All functional groups and summer annuals	*A. menziesii*, *C. setigerus*, *H. fasciculata*, *L. californica*, *L. platyglossa*, *P. erecta,*
7)	Tall winter annual forbs and summer annuals	*A. menziesii*, *C. setigerus*, *H. fasciculata*, and *L. platyglossa*
8)	Short winter annual forbs and summer annuals	*C. setigerus*, *H. fasciculata*, *L. californica*, and *P. erecta*
9)	Perennial winter forbs and summer annuals	*C. setigerus*, *H. fasciculata*, *H. villosa*, and *L. filaginifolia*
	Plots with Native Forbs and Invasive Species	
10)	All functional groups and invasive species	*A. menziesii*, *B. diandrus, L. californica*, *L. platyglossa*, *P. erecta*
11)	Tall winter annual forbs and invasive species	*A. menziesii*, *B. diandrus*, and *L. platyglossa*
12)	Short winter annual forbs and invasive species	*B. diandrus*, *L. californica*, and *P. erecta*
13)	Perennial winter forbs and invasive species	*B. diandrus*, *H. villosa* and *L. filaginifolia*
14)	All functional groups with summer annuals and invasive species	*A. menziesii*, *B. diandrus, C. setigerus*, *H. fasciculata*, *L. californica*, *L. platyglossa*, *P. erecta,*
15)	Tall winter annula forbs with summer annuals and invasive species	*A. menziesii*, *B. diandrus*, *C. setigerus*, *H. fasciculata*, and *L. platyglossa*
16)	Short winter annual forbs with summer annuals and invasive species	*B. diandrus*, *C. setigerus*, *H. fasciculata*, *L. californica*, and *P. erecta*
17)	Perennial winter forbs with summer annuals and invasive species	*B. diandrus*, *C. setigerus*, *H. fasciculata*, *H. villosa*, and *L. filaginifolia*

Table 1.2 – Mean density, percent cover, and biomass for native and invasive species in different functional group plots for the 2013-14 growing season. ANOVAs were conducted to assess differences in native and invasive plant composition between functional group treatments. Significance was determined at α=0.05.

	Density/m2					Percent Cover					Biomass (g/m2)				
	Control	All functional groups	Tall Forbs	Short Forbs	P-value	Control	All functional groups	Tall Forbs	Short Forbs	P-value	Control	All functional groups	Tall Forbs	Short Forbs	P-value
Native Species															
A. menziesii	9.7	24.3	54.0	--	**0.014**	3.2	10.8	34.2	--	**0.008**	54.2	126.1	336.5	--	**0.007**
L. platyglossa	0.8	16.0	5.0	--	NS	0.5	3.8	2.0	--	NS	3.1	21.1	10.9	--	NS
L. californica	1.0	0.8	--	23.3	**0.025**	0.2	0.3	--	6.8	**0.008**	0.3	0.4	--	13.7	**0.008**
P. erecta	3.1	58.4	--	93.5	**0.016**	0.8	14.2	--	30.5	**0.024**	1.0	29.1	--	64.4	**0.025**
Native Species w/ Invasive Species															
A. menziesii	9.7	30.7	27.4	--	**0.041**	3.2	8.0	10.6	--	**0.049**	54.2	101.4	124.7	--	**0.047**
L. platyglossa	0.8	7.4	14.0	--	**0.011**	0.5	2.9	4.7	--	**0.050**	3.1	16.5	24.0	--	**0.021**
L. californica	1.0	20.6	--	15.5	NS	0.2	2.6	--	2.3	NS	0.3	5.1	--	4.3	NS
P. erecta	3.1	22.4	--	32.0	NS	0.8	3.6	--	6.3	**0.008**	1.0	6.6	--	12.0	**0.010**
Invasive Species					NS					NS					NS
B. diandrus	68.3	34.4	42.0	15.0	NS	24.0	17.0	14.8	10.75	NS	548.3	344.3	299.5	196.4	NS
E. cicutarium	153.6	165.8	118.8	162.75	NS	44	57	43.4	50.3	NS	368.3	286.1	281.4	325.1	NS

PLANT-SOIL FEEDBACKS AND COMPETITIVE INTERATIONS BETWEEN INVASIVE *BROMUS DIANDRUS* AND NATIVE FORB SPECIES

ABSTRACT

Feedback between plant and soil microbial communities plays a key role in plant invasions. I examined feedback in native and invasive plants growing in monoculture and mixture, to determine soil microorganisms' role in *Bromus diandrus* invasion. Four native forb species were grown in monoculture and in competition with *Bromus* and with different microbial inocula. Inoculum consisted of 20g of soil collected from the rhizosphere of native or invasive plants used to create treatments of (1) whole soil, (2) filtrate containing non-mycorrhizal microbes, and (3) arbuscular mycorrhizal fungi (AMF) spores. Native species in monoculture experienced neutral to positive feedback with whole soil and filtrate inoculum. Feedback in *Bromus* grown in monoculture varied in direction and magnitude with different soil microbial fractions. Fine AMF (*Glomus tenue*) in filtrate inoculum appeared to cause observed positive feedback effect in native and invasive species, even with pathogenic fungi in roots. Feedback in mixture was more positive than in monoculture for some species. My study highlights the difficulty of extending feedback results in monoculture to the community level, and the importance of fine AMF, which has received little attention, interacting with pathogens in plant invasion.

INTRODUCTION

While many mechanisms have been proposed to explain the success of invasive species, plant-soil feedback has been widely proposed and tested over the past two decades (Klironomos 2002, Callaway et al. 2004b, van Grunsven et al. 2007, Batten 2008, van der Putten et al. 2013). Plant- soil feedback is defined as plant-influenced changes to the soil microbial community that then positively or negatively affects subsequent plant growth (Bever 1994, Bever et al. 1997). Much of the plant-soil feedback research has approached soils as a black box, and explanations of invasiveness assume the role of either soil-borne pathogens or mutualists though they are seldom observed (reviewed in van der Putten et al. 2013, but see Klironomos 2002 and Callaway et al. 2011).

Biogeographical comparisons of plant species often detect more negative effects of soil biota from plants' native vs. non-native ranges (Callaway et al. 2004b, Callaway et al. 2011). Invasive species may establish in a novel environment due to a release from soil-borne pathogens (Keane and Crawley 2002, Bezemer and Van der Putten 2007, Kardol et al. 2007, Reinhart et al. 2010). Alternatively, invasive species can alter the soil biota in invaded ranges creating positive feedback effects that promote invasion (Richardson et al. 2000, Volgelsang et al. 2009). Associated native species form either positive or negative feedback (Klironomos 2002), and the direction of the feedback may affect interspecific competition and plant community composition.

Both feedback effects and the potential role of competitive interactions are significant in plant invasion but seldom studied together (Hodge and Fitter 2013). Soil mutualists (Callaway et al. 2004b) and pathogens (van der Putten and Peters 1997) affect competitive interactions, and in the context of competition feedback effects may change in direction and magnitude (Shannon et al. 2012). Stabilizing mechanisms of species coexistence would suggest plant species in intraspecific competition experience a greater negative growth response than in interspecific competition (Chesson 2000, Casper and Castelli 2007). Therefore, invasive species may experience more negative feedback effects over time as they continue to dominate a plant community. However, Casper and Castelli (2007) found no evidence that intraspecific competition results in greater negative growth response, and the combined effects of competition and the strength of the growth response was different among species. This suggests that plant responses to soil biota when grown in intraspecific competition cannot adequately predict plant responses to soil biota when grown in interspecific competition (Allen and Allen 1984). Studies examining soil biota in invasions need to examine growth responses of the invasive species both in intraspecific competition and in competition with the native species it displaces.

Bromus diandrus is a Mediterranean annual grass invading much of the remaining coastal sage scrub and native forbland communities in southern California (Barbour et al. 2007, Minnich 2008). Invasive grasses have been shown to alter soil dynamics that contributes to their overall success in coastal sage scrub (Dickens et al. 2013), and host a different assemblage of arbuscular mycorrhizal fungi (AMF) from native plants (Hawkes

et al. 2006, Sigüenza et al. 2006, Busby et al. 2013). Exotic grasses in coastal sage scrub are predominately infected with fine AMF, often identified as *Glomus tenue*, whereas native shrubs they displace are infected mainly with coarse AMF and infection by fine AMF is infrequent (Sigüenza et al. 2006). *Glomus tenue*, the fine AMF, has been reported to infect numerous grass species (Molina et al. 1978, Powell 1979, Rabatin et al. 1993) and be more frequent in pioneer plants (Blasche 1991). It is commonly found in a wide range of soils including agricultural and forest soils, at a wide range of altitudes, often recorded in lowlands and high mountain soils (Abbot and Robson 1977, Molina et al. 1978, Blaszowksi 1994), and is especially common in degraded soils (Gucwa-Przepióra et al. 2013). While some studies suggest the fine endophyte is the main root colonizer in the absence of other AMF, the ecology of the fine AMF is poorly understood and its role in invasion is virtually unstudied (but see Sigüenza et al. 2006).

The influence of *Bromus diandrus* on the soil community and subsequent impacts on native forb growth and interspecific competition is unknown. I examined the role of soil microbial feedbacks in the competitive dominance of the invasive grass *Bromus diandrus*. More specifically, I examined (1) plant-soil feedback effects from native and invasive plants on conspecific and interspecific growth, (2) tested different microbial fractions to evaluate which groups of fungi influence plant-soil dynamics and, (3) determined whether native or invasive inoculum affect growth and competition between *Bromus diandrus* and native forbs.

MATERIAL AND METHODS

Study Site

Soils for this study were collected at Riverside County Habitat Conservation Agency lands near Lake Mathews, in Riverside, CA (33°36'29.80 N, 117°02'00.81 W) in September 2012. The site is abandoned citrus agriculture that was formerly coastal sage scrub (CSS) and annual forbland (Minnich 2008), and is currently dominated by the exotic annual grass *Bromus diandrus*. Citrus trees were removed some five years prior to our study when the land was acquired as a conservation reserve. Bulk soil to be used as a greenhouse growth medium was collected in an adjacent 2 ha native CSS community. Soils from both the citrus agricultural site and the adjacent CSS site are in the Porterville cobbly clay series (Nelson et al. 1919). The soil was cut fifty percent with silica sand to improve drainage (a common practice for inoculum studies in fine-textured soil, e.g., Johnson et al. 2008), steam-sterilized for 24 hours, held at room temperature for 24 hours, and sterilized for another 24 hours. The resulting soil contained total KCl-extractable N (NO_3^--N plus NH_4^+-N) of 17.0 μg/g soil, and 18.1 μg/g bicarbonate-extractable P. This soil mix was placed into 800ml Conetainer® pots, and seed mixes and soil inocula with or without biota as described below were added to pots.

Soils and Inoculum Material

Inoculum soil for the greenhouse experiment was collected directly from the field to assure that field-cultured microbial species were present. Native CSS inoculum was

taken in the 2 ha remnant stand from underneath fifteen *Artemisia californica* shrubs, whose understory consists of a mixture of native annual forb species including all of the native annuals in this study, to a depth of high fine root activity (10 cm) and mixed. Therefore, our native inoculum contains the soil microorganisms from a natural CSS community where shrub and forb species co-occur in a matrix, and changes to the soil from that matrix may have consequences for the growth or fitness of the species within the matrix. Invasive inoculum was collected underneath fifteen *Bromus diandrus* plants from the abandoned citrus orchard. By collecting inoculum directly from the field I assured that organisms that represented the legacy of abandoned citrus agriculture, including oomycetes and *Fusarium* spp., and native CSS were included in the inoculum (Allen et al. 1993).

Soil feedback in native versus invasive plants was determined using additions of soil inoculum with or without soil biota from different microbial fractions. Seven soil microbial fractions were created from 20 grams of inoculum soil for each replicate pot: 1) sterile soil, 2) native whole soil, 3) invasive whole soil, 4) native filtrate, 5) invasive filtrate, 6) native AMF spores, and 7) invasive AMF spores. Twenty grams of soil per pot were passed through a 2mm sieve for whole soil inoculum, or a 20µm sieve to create a filtrate that excludes AMF spores > 20 µm and includes potential pathogens (Klironomos 2002). AMF spores were collected using the sucrose extraction method (Allen et al. 1979), and were surface sterilized with 5.25% sodium hypochlorite. An average number of 435 AMF spores occurred in 20g of inoculum from native CSS species, whereas 239 spores were found in 20g of *Bromus* inoculum. Pots each received 20g of steam-sterilized

inoculum from the other source and sterile soil received 20g of steam-sterilized inoculum soil from each of the two inoculum sources (40g total) to balance nutrients in soil from native and former agricultural land.

Greenhouse Experimental Design

In a controlled greenhouse environment, four native forbs and the exotic annual grass *Bromus diandrus* were grown from seed in monocultures in the seven soil treatments described above for six weeks (n=10). Additionally, native forbs were grown in competition with *Bromus* in native and invasive whole soil inoculum, and sterile soil (n=10). Based on vegetation surveys completed in 2010 at Lake Mathews (Allen unpublished), I selected two common forbs (*Amsinckia menziesii* and *Layia platyglossa*) and two uncommon forbs (*Plantago erecta* and *Lasthenia californica*). Seeds of the native forb species were from regional collections from S&S Seed Co. (Carpinteria, California), and seeds of *Bromus diandrus* were collected at Lake Mathews in September 2011. Seeds of all five species were planted and thinned to two individuals of the same species for monocultures, and one native forb individual with one *Bromus* individual for mixtures. The resulting 470 pots were arranged in a complete randomized design to control for potential temperature gradients in the greenhouse.

Microbial Assessment for Feedback

After 6 weeks, plants were harvested for aboveground biomass and root biomass. Biomass was determined after drying at 60° C for 48 hours. Plant-soil feedback was

calculated in whole soil inoculum, filtrate, and AMF spore treatments using the following equation: soil feedback = [aboveground biomass of plant grown in inoculum fraction – aboveground biomass of plant grown in sterile soil]. Dried root biomass was rehydrated and mycorrhizal/non-mycorrhizal fungi colonization was assessed (prior observations showed that drying did not change percent colonization of mycorrhizal or pathogenic fungi). To assess fungal colonization, roots 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). Percent colonization was estimated using a modified magnified intersection method (McGonigle et al. 1990). Roots were mounted in PVLG on microscope slides and 80 intercepts per replicate were observed at 400X magnification. Root fragments were examined for coarse AMF hyphae, fine endophytic AMF hyphae, pathogenic/saprophytic hyphae, oomycete hyphae, vesicles, and arbuscules. Coarse AMF hyphae are aseptate, 2-10 μm in diameter, and characterized by defining features such as dichotomous branching at a 60 degree angle and knobby hyphal walls that stain dark (Rillig et al. 1999, Sigüenza et al. 2006). Fine endophyte AMF have thinner hyphae, <2 μm in diameter, and lightly stained walls in these roots (Sigüenza et al. 2006). Hyphae of the Ascomycota are characterized as having septa at regular intervals and sometimes staining blue while other times non-staining. Fungi of the Ascomycota range from purely saprophytic to obligate pathogens, and include important plant pathogens such as *Fusarium* sp. (Webster and Weber 2007). Previous culturing from this field site identified two *Fusarium* species *Fusarium equiseti* and *Fusarium pseduograminerarum* (Hilbig, unpublished). Both species are known pathogens.

Dikaryotic hyphae of the Basidiomycota are characterized as having distinct clamp connections, or lateral bulges in the hyphae, at regular intervals (Webster and Weber 2007). Oomycetes are morphologically identified by coenocytic hyphae with walls that lack chitin and therefore fail to stain with trypan blue. Additionally, oomycetes are determined morphologically by distinct lemon-shaped sporangia, 10-20μm in width (Webster and Weber 2007).

Statistical Analysis

Biomass data were analyzed using separate one-way ANOVA for each species, with soil treatment as a fixed factor. Soil treatments were compared using least significant difference ($L.S.D._{0.05}$). All data were checked for homogeneity of variances using Levene's tests, and for normality using the Shapiro-Wilk test. For all species, total biomass data was ln transformed to meet the assumptions of normality for ANOVA. Percent root colonization data failed to meet the normality assumption even after a log transformation, and were analyzed using Kruskal-Wallis rank sum test for each species with soil treatment as a fixed factor. All statistical analyses were performed using R version 3.0.2 (R Development Core Team 2013).

Feedback was modeled in a Bayesian framework to incorporate different variances among species-soil treatment combinations. Biomass within each species-inoculum treatment was modeled using a normal distribution and its own variance. Feedbacks were calculated for each species within the model as $\text{Aboveground Biomass}_{\text{microbial fraction}} - \text{Aboveground Biomass}_{\text{sterile}}$. . P values were calculated as the

probability that the posterior probability distributions of these feedbacks overlapped zero, with significant values ≤ 0.05 (corresponding to 95% credible intervals that did not overlap zero). All mean and variance parameters were given non-informative priors, models were run for 20,000 iterations, and convergence was assessed by visual inspection of three independent chains after a brief burn-in period. Models were fit using OpenBUGS version 3.2.2 rev 1063 called from R using the R2OpenBUGS package (R Developing Core Team, Sturtz et al. 2005). Feedback was calculated using aboveground biomass due to the difficulty of separating root biomass by species when plants were grown in mixture. In monoculture, where root biomass was measured, I compared feedback calculated with total biomass to feedback calculated with aboveground biomass. Feedback did not change significantly in direction in any case, and in both filtrate and whole soil treatments significant feedback was observed in the same species regardless of the biomass data used. In AMF inocula treatments 4 of the 10 species treatment combinations shifted from trending to significant or vice versa. I therefore used aboveground biomass so that feedback could be compared between monoculture and mixture.

Statistical comparisons of feedback in monoculture and mixture for each species-inoculum treatment were done by modeling Difference = (Aboveground $Biomass_{microbial\ fraction}$ – Aboveground $Biomass_{sterile}$) in monoculture - (Aboveground $Biomass_{microbial\ fraction}$ – Aboveground $Biomass_{sterile}$) in competition. P values were calculated as the probability that the posterior probability distributions of feedback differences overlapped zero, with significant values ≤ 0.05 (corresponding to 95% credible intervals that did not overlap

zero). All mean and variance parameters were given non-informative priors, models were run for 20,000 iterations, and convergence was assessed by visual inspection of three independent chains after a brief burn-in period. Models were fit using OpenBUGS version 3.2.2 rev 1063 called from R using the R2OpenBUGS package (R Developing Core Team, Sturtz et al. 2005).

RESULTS

Monocultures

Aboveground biomass of *Amsinckia* and *Plantago* did not differ significantly by soil treatment when grown in monoculture (Fig 3.1a & 1d). *Lasthenia* grown in soil with native AMF spores and invasive whole soil inocula had greater aboveground biomass than *Lasthenia* grown with sterile soil, native whole soil or invasive AMF spores inocula ($F= 5.231$, $P <0.0001$; Fig 3.1b). Similarly, *Layia* grown in soil with native whole soil inoculum and native AMF spores had greater aboveground biomass than *Layia* grown in sterile soil and filtrate from native inoculum ($F=4.509$, $P<0.001$; Fig 3.1c). Aboveground biomass of *Bromus* was smaller when plants were grown with native filtrate inocula than all other soil treatments except invasive AMF spores inocula ($F=5.877$, $P<<0.0001$; Fig 3.2a).

Competition with Bromus diandrus

Across all native species, plant biomass was smallest in sterile soils when grown in competition with *Bromus* (Fig 3.1e-h). *Amsinckia, Layia* and *Plantago* grown in competition with *Bromus* had increased aboveground biomass with whole soil inoculum from both inoculum sources compared to sterile treatments (Fig 3.1e, 3.1g & 3.1h). *Lasthenia* had greater aboveground biomass in invasive than native whole soil inoculum (Fig 3.1f; $F=8.78$, $P=0.0014$). *Bromus* aboveground biomass was significantly greater in whole soil inocula than sterile soil when grown with all native forb species, except *Lasthenia* (Fig 3.2b-e).

Soil Feedback

Calculated feedback for each species is graphically represented with absolute values (Fig 3.3 & 3.4) and biomass was not standardized for comparisons across species. Feedback in all four native species grown in monoculture experienced neutral to positive feedback (Fig 3.3). Significant positive feedback was observed in both *Lasthenia* and *Layia* when grown with native AMF spores ($P=0.007$, $P=<0.0001$), invasive filtrate ($P=0.005$, $P=0.017$), and native whole soil ($P=0.047$, $P<0.0001$). *Lasthenia* also had a positive feedback when grown with native filtrate ($P=0.012$) and invasive whole soil inoculum ($P=0.010$). *Bromus* had a positive feedback when grown with native AMF spores ($P=0.003$) and invasive filtrate ($P=0.042$), and negative feedback when grown with native filtrate ($P=0.006$). *Amsinckia* and *Plantago* had no significant feedback across all soil treatments at $\alpha=0.05$.

In competition with *Bromus*, *Layia* and *Plantago* had significant positive feedback when grown with whole soil inoculum from both inoculum sources (native whole soil: P<<0.001, P<<0.001; invasive whole soil P=0.009, P<<0.001 respectively). Calculated feedback with invasive whole soil inoculum was stronger when plants were grown in mixture than in monoculture for both *Layia* and *Plantago* (P=0.048, P=0.023; Fig 3.4a). *Amsinckia* had a positive feedback in invasive whole soil only (P=0.002; Fig 3.4a). *Bromus* grown with *Amsinckia* and *Plantago* had positive feedback with whole soil inoculum from both sources (Fig 3.4b). These feedbacks were significantly stronger than the positive feedback observed in *Bromus* grown in monoculture under the same soil conditions (P=0.013 and P=0.015 for *Bromus* with *Amsinckia,* and P<<0.001 and P=0.013 for *Bromus* with *Plantago,* in native and invasive whole soil respectively).

Percent Root Colonization

Both coarse and fine AMF hyphae were found colonizing the roots of all five species, although native forb species were colonized more by fine AMF hyphae when grown with invasive inoculum and more heavily colonized by coarse AMF in native inoculum (Table 2.1, Fig 3.5). For example, in *Amsinckia* grown with native whole soil inoculum 72% of the total mycorrhizal colonization was by coarse AMF compared to 75% of the total mycorrhizal colonization by fine AMF colonization when grown with invasive whole soil inoculum. Similarly, the majority of root colonization (65%) of *Lasthenia* grown with native whole soil inoculum was by coarse AMF, whereas in the invasive whole soil inoculum 70% of the total mycorrhizal colonization came from fine

AMF colonization. *Bromus* had the lowest total AMF root infection on average across all five species, and was predominately infected by fine AMF in both native and invasive inoculum (Fig 3.5). It had significantly greater colonization of fine AMF when grown with invasive whole soil inoculum and invasive AMF spore inoculum compared to all other soil treatments ($H=16.3713$, $df=6$, $P= 0.0119$; Table 2.1). *Layia* had the highest total percent AMF root colonization across all five species, with up to 53% of roots infected when grown with invasive whole soil (Table 2.1). Similarly, high percent AMF infection was found in *Layia* grown with invasive AMF spores, invasive filtrate, and native AMF spores (about 30% each treatment). Individuals of *Plantago* had high percent root colonization of AMF when grown with AMF spores from both inocula sources and whole soil inoculum from both sources (Table 2.1). Filtrate treatments from both inocula contained the fine AMF (spores <20μm), and *Layia* grown with invasive filtrate had up to 30% of roots colonized by fine AMF. Virtually all of the colonization was by AM hyphae, with no more than 2% vesicles and no arbuscules in any treatment.

In every observation, hyphae that morphologically appeared to be ascomycetes were the dominant form of non-mycorrhizal hyphae. Overall, the greatest colonization by non-mycorrhizal fungi occurred in species grown in invasive whole soil inoculum. *Lasthenia* had the highest percent non-mycorrhizal fungi colonization (30.1%) when grown in invasive whole soil inoculum. Root colonization of *Amsinckia* by non-mycorrhizal fungi was significantly higher in invasive whole soil inoculum than all other soil treatments ($H=23.88$, $df=6$, $P<0.001$;Table 2.1). High percent root colonization by non-mycorrhizal fungi was found in individuals of *Plantago* grown with invasive whole

soil, native whole soil, and invasive filtrate. Similarly, a high percentage of non-mycorrhizal fungi were found colonizing the roots of *Bromus* in whole soil inoculum from both inocula sources. A low percentage of oomycete hypha was found in the roots of the four forb species, but not *Bromus*, in the invasive AMF spore inoculum and invasive whole soil inoculum. *Layia* had the greatest infection of oomycetous hyphae among the forbs (Table 2.1). There was some contamination in sterile treatments, but oomycete hyphae were never found colonizing the roots of plants grown with native inoculum fractions.

DISCUSSION

Co-existence theory predicts that co-occurring species experience negative feedback that prevents species dominance and contributes to ecosystem stability (Chesson 2000, Bever et al. 2012, Reinhart 2012). Invasive species often benefit from positive feedback (Richardson et al. 2000) while native species experience negative feedback contributing to an invasive species overall dominance. However, in our study all four native species experienced neutral to positive feedback. The unexpected positive feedback of native species may be explained by their annual life history. Mixed populations of native annual forbs in the understory of coastal sage scrub change in abundance annually with fluctuating rainfall characteristic of semi-arid Mediterranean climates (Heady 1958). Inoculum soil contained inputs from a mixture of native annual and shrub roots, including the annual species tested. The predominant negative feedback

of native species described in other studies (Kulmatiski et al. 2008) may not occur in unstable populations of annuals. The observed positive feedback could contribute to the instability of this invaded annual system where the competitively superior invasive species experiences positive feedback leading to its dominance.

Fine AMF may also contribute to the unexpected positive feedback in native forbs and *Bromus*. The use of microscopy and morphological identification of fungal groups revealed that the soils of our *Bromus*-dominated, recently abandoned agricultural site have a high load of fine AMF, often identified as *Glomus tenue*. AMF are obligate mutualists (Smith and Read 2008). Therefore, the shift in AMF colonization in native forbs from coarse to fine AMF when grown with native and invasive inoculum respectively suggests that culturing of fine AMF by *Bromus* shifts the mycorrhizal community and de-stabilizes the system. Other studies have demonstrated significant shifts in AMF communities following invasion (Mummey and Rillig 2006), and these shifts may confer a competitive advantage to the invasive species.

Little is known about the taxonomy, physiology and ecology of the fine AMF, although a few studies examining plant responses to infection by fine AMF exist (Powell 1979, Rabatin 1993, Sigüenza et al. 2006, Zubek et al. 2009). Our results suggest that the fine AMF is important in the success of *Bromus* through neutralizing negative impacts of potential pathogens. This is demonstrated through positive feedback in *Bromus* when grown in soil inoculated with the invasive filtrate treatment described above and negative feedback when grown in soil inoculated with the native filtrate. Feedback is the net combination of mutualists and pathogens, and any potential negative impact of non-

mycorrhizal fungi may be offset by positive responses to fine AMF. In native filtrate, higher percent root colonization by pathogens than by fine AMF resulted in a significant negative feedback. Whereas, when fine AMF infection was greater than pathogen infection in invasive filtrate, *Bromus* experienced positive feedback. Other studies have demonstrated positive plant growth responses in native (Powell 1979) and invasive (Sigüenza et al. 2006) species to *Glomus tenue*. Positive feedback in *Bromus* grown with native AMF spores is due to the combination of coarse and fine AMF. While I predict *Bromus* would benefit from a positive feedback with invasive AMF spores due to infection by fine AMF, high within-treatment variation results in a non-significant neutral feedback that overlaps zero. Variation in growth within treatment cannot be explained by differences in fungal colonization, but may be related to factors not explicitly studied here such as seed size or germination timing. Further understanding of the effects of fine AMF on plant growth will require plants to be grown with single species of AMF.

At this point I do not know to what extent the fine AMF has been introduced with exotic grasses, or if the fine AMF is locally native and increasing in abundance because the most abundant plant species is culturing it. While a dominant native CSS shrub, *Artemisia californica,* had little fine AMF colonization in the field or greenhouse, even when grown in mixtures with exotic grasses (Sigüenza et al. 2006), in our study all four native forbs were colonized by fine AMF and experienced neutral to positive feedback. However, in competition with *Bromus,* native forb species, with the exception of *Amsinckia,* experienced reduced biomass relative to intraspecific competition regardless of the inoculum source. Whole soil inoculum resulted in greater forb biomass than sterile

soil when in competition with *Bromus,* suggesting that though native forbs are poor competitors with *Bromus* AMF may partially alleviate the negative competitive effects of *Bromus*. The fact that *Amsinckia* does not have reduced biomass in mixture with *Bromus* suggests that it is a better competitor with the invasive grass than other natives, and in fact *Amsinckia* is more abundant than other native annuals at our site (unpublished observations) as well as at other invaded California annual grasslands (Pantone et al. 1995).

The high frequency of fine AMF in our soils collected from the rhizosphere of *Bromus* demonstrates that the traditional methods in plant-soil feedback studies to partition out non-mycorrhizal fungi in a microbial filtrate by using a 20μm sieve (Klironomos 2002, Agrawal et al. 2005, Kardol et al. 2007, Callaway et al. 2011) may not always work as expected. Fine AMF spores have been observed to be as small as 10 μm in diameter (personal observation, Sigüenza et al. 2006), and thus in our study the filtrate treatment allowed passage of both fine AMF and possible pathogens. Most studies report using 100X magnification to assess AMF (McGonigal and Fitter 1990), but because of the small diameter and poorly staining cell walls of fine AMF hyphae in our roots, they must be observed at 400X. It is possible that fine AMF is more prevalent than published literature would suggest and its ecological importance in plant community composition warrants further investigation.

Our study focused on soil fungi in *Bromus* invasion, although other microbes might affect plant-soil feedback including oomycetes, microfauna, and bacteria. Perhaps the most unexpected finding of this study was the presence of oomycetes in the invasive

AMF spore inoculum. The field site is a former citrus orchard, and citrus is known for high incidence of root diseases (Kosola et al. 1995). I are not aware of reports of a high incidence of oomycetes in roots of native plants. In fact, they are thought to be highly host-specific, and not expected to infect the roots of native plants. The occurrence of oomycete hyphae in the invasive AMF spore treatment for some species may explain the neutral feedback, as the negative growth responses of known oomycete pathogens are balanced by the positive responses to AMF. Nematodes are often the most abundant microfauna, and can be readily observed on root surfaces or in sucrose spore extracts (Persmark et al. 1992). I did not observe nematodes in our sucrose spore extracts or microscope slides, therefore they are likely not abundant in these soils. Plant growth-promoting bacteria could result in positive growth responses in plants (Çakmakçi et al. 2006), but bacteria species would be similar among all fractions except sterilized soils and could not adequately explain different growth response in plants to different soil fractions. Thus our results are best explained by the balance of AMF and potential pathogens.

Lastly, I observed plant-soil feedback from fungi in interspecific competition and intraspecific competition. Feedback changed in magnitude in the context of competition, and in some species the feedback in mixture was more positive than the feedback in monoculture. Competition for mutualists in intraspecific competition may be stronger than in interspecific competition due in part to niche differentiation of AMF symbiosis. *Bromus* was predominately infected with fine AMF whereas native forbs were infected with both fine and coarse AMF. Thus individuals in intraspecific competition may

experience greater competition for symbionts than individuals grown in interspecific competition, which may lead to a more positive feedback in interspecific competition. Others have suggested that in interspecific competition plants may benefit from common mycelium networks (Callaway et al. 2004a), but the mechanism behind shifts in the magnitude of feedback with competition is still poorly understood. Our study further demonstrates the difficulty of extrapolating the effects of feedback from monocultures to competition, and extending plant-soil feedback studies to the community assembly framework. A better mechanistic understanding of microbe-root interactions in monoculture and mixture will be needed to differentiate the effects of competition and feedback in plant-plant interactions.

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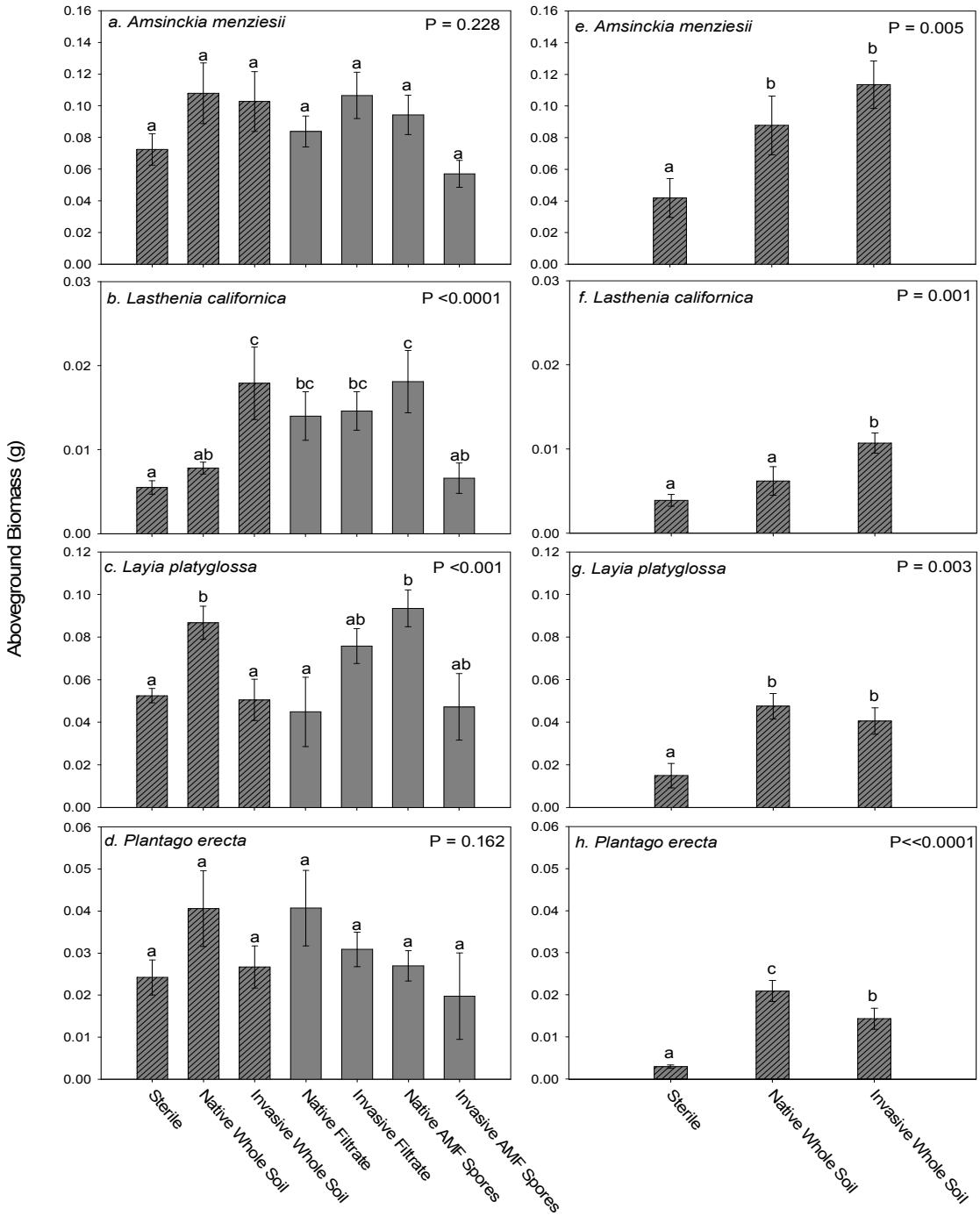

Figure 2.1 - Aboveground biomass of native forbs in monoculture (a-d) and mixture with *Bromus* (e-h) grown under different soil inoculum conditions. Patterned bars represent soil treatments that occur in both monoculture and mixture. Significance was determined at α=0.05.

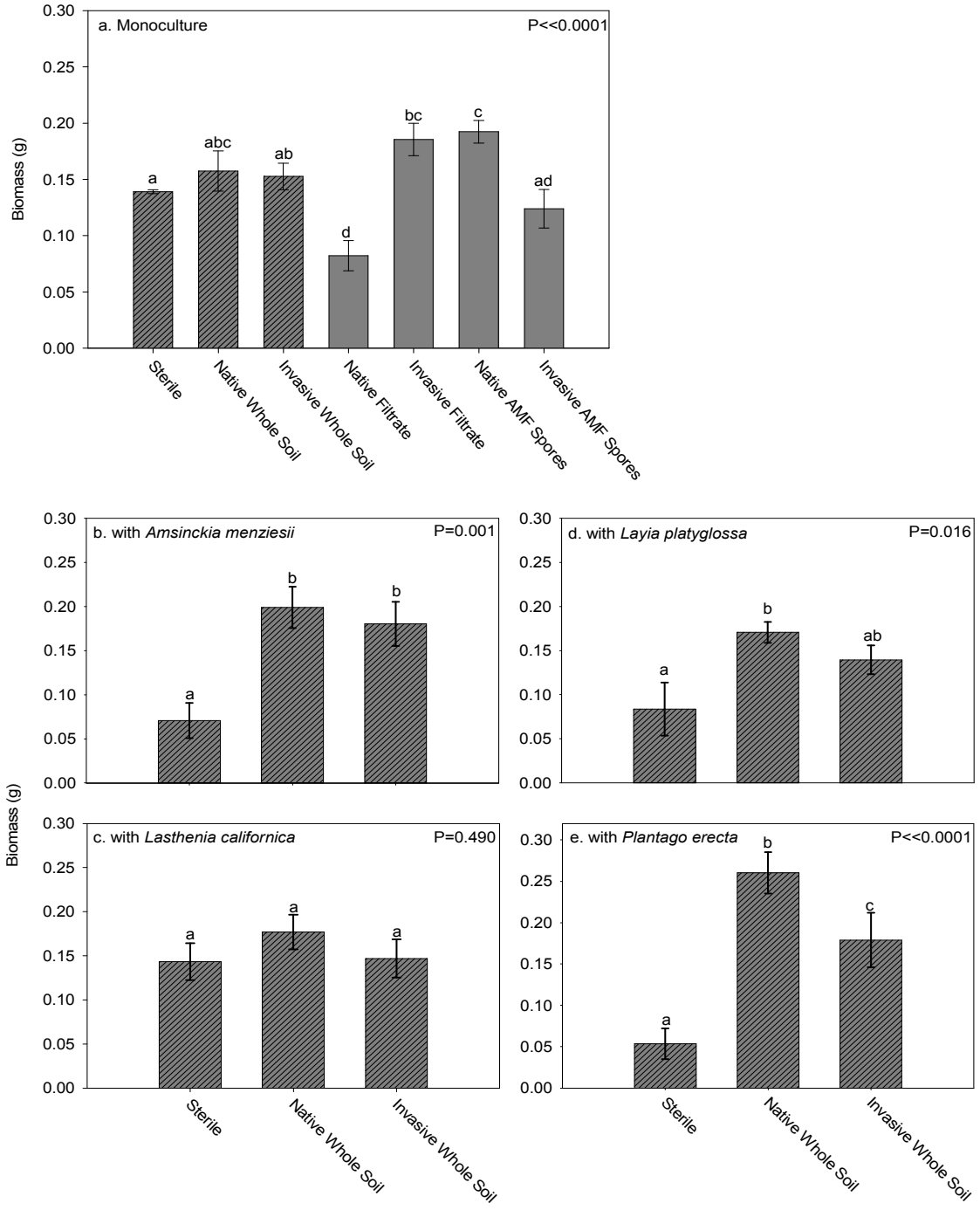

Figure 2.2 - Aboveground biomass for *Bromus* grown in monoculture (a) and in mixture with four native forb species (b-e) in different soil microbial fractions analyzed using separate one-way ANOVA. Patterned bars are those soil treatments that occur in both monoculture and mixture. Significance was determined at $\alpha=0.05$.

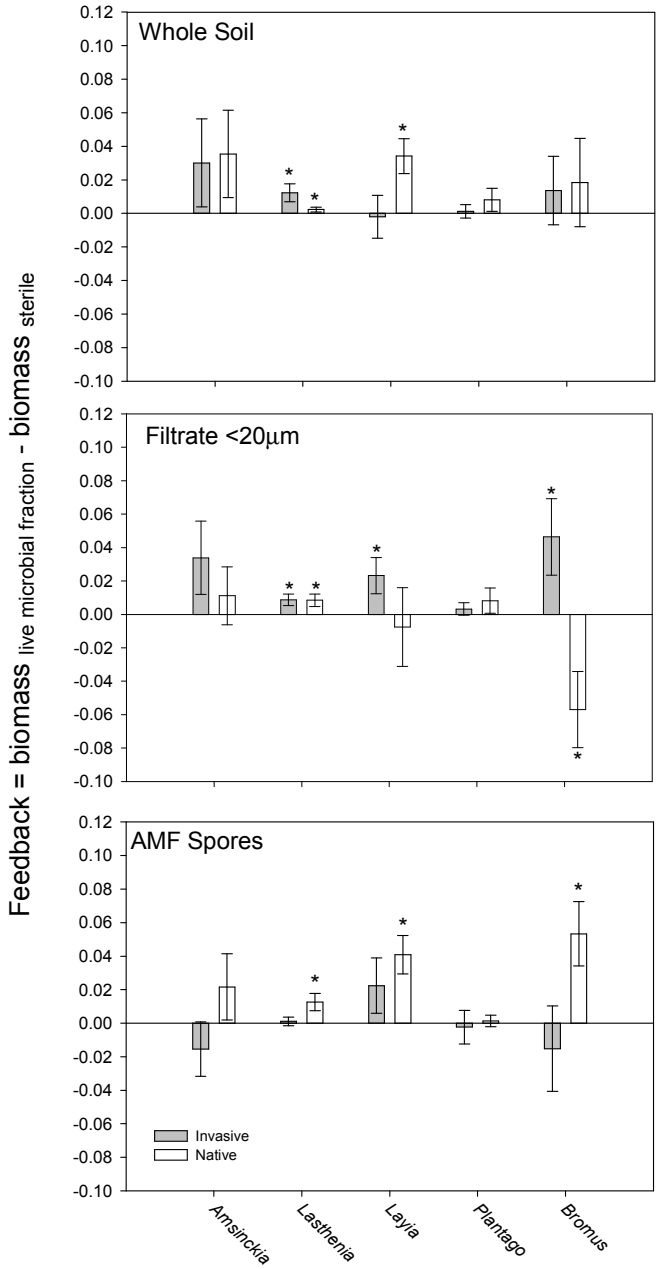

Figure 2.3 - Feedback calculated for all five species grown in monoculture by [aboveground biomass of plant grown in inoculum fraction – aboveground biomass of plant grown in sterile soil]. The open bars are feedback calculated from microbial fractions collected from the rhizosphere of *Artemisia californica*. The gray bars are feedback calculated from microbial fractions collected from the rhizosphere of *Bromus diandrus.* Asterisks represent significant feedback at α=0.05.

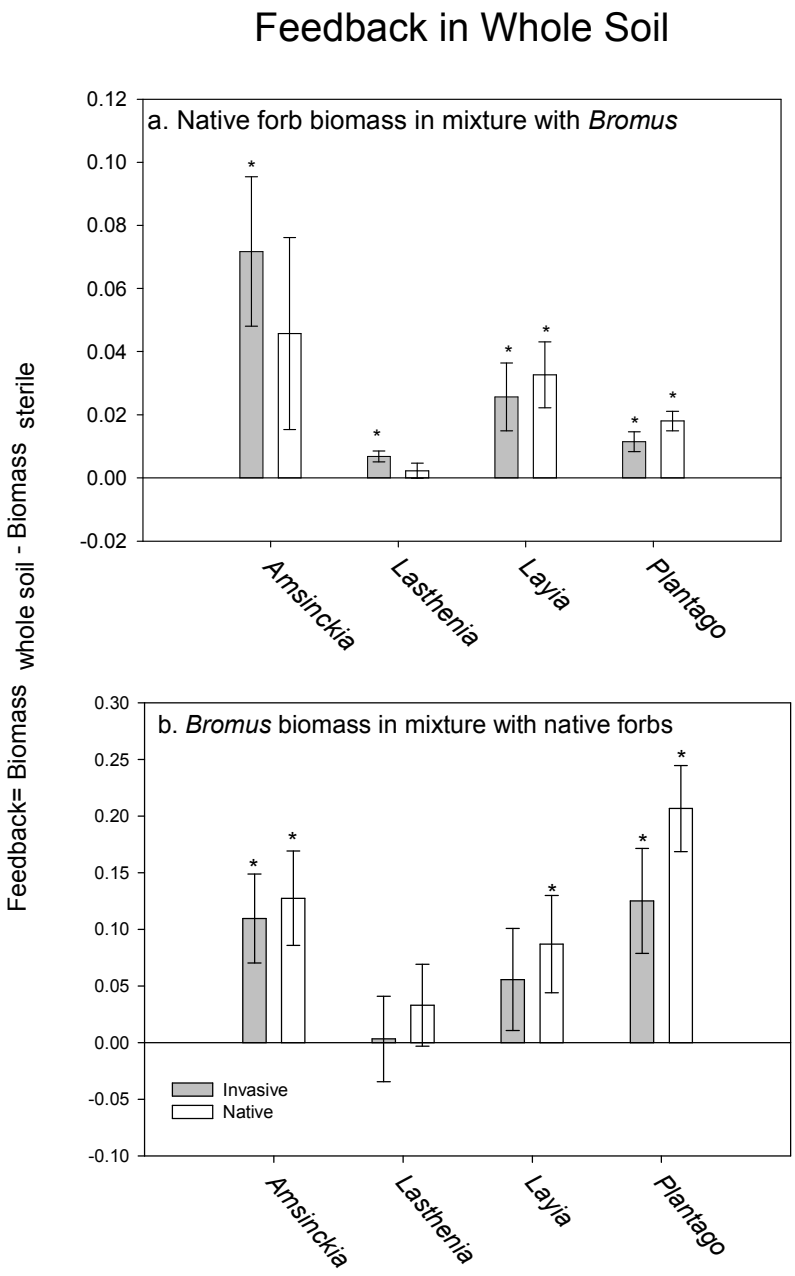

Figure 2.4 - Feedback calculated for four native forb species grown in mixture with *Bromus* (a) as [aboveground biomass of in whole soil – aboveground biomass in sterile soil]. *Bromus diandrus* feedback in mixture with native forbs (b) calculated by [aboveground biomass of *Bromus* in whole soil – aboveground biomass of *Bromus* in sterile soil]. The open bars are feedback calculated from microbial fractions collected from the rhizosphere of *Artemisia californica*. The gray bars are feedback calculated from microbial fractions collected from the rhizosphere of *Bromus diandrus.* Asterisks represent significant feedback at α=0.05.

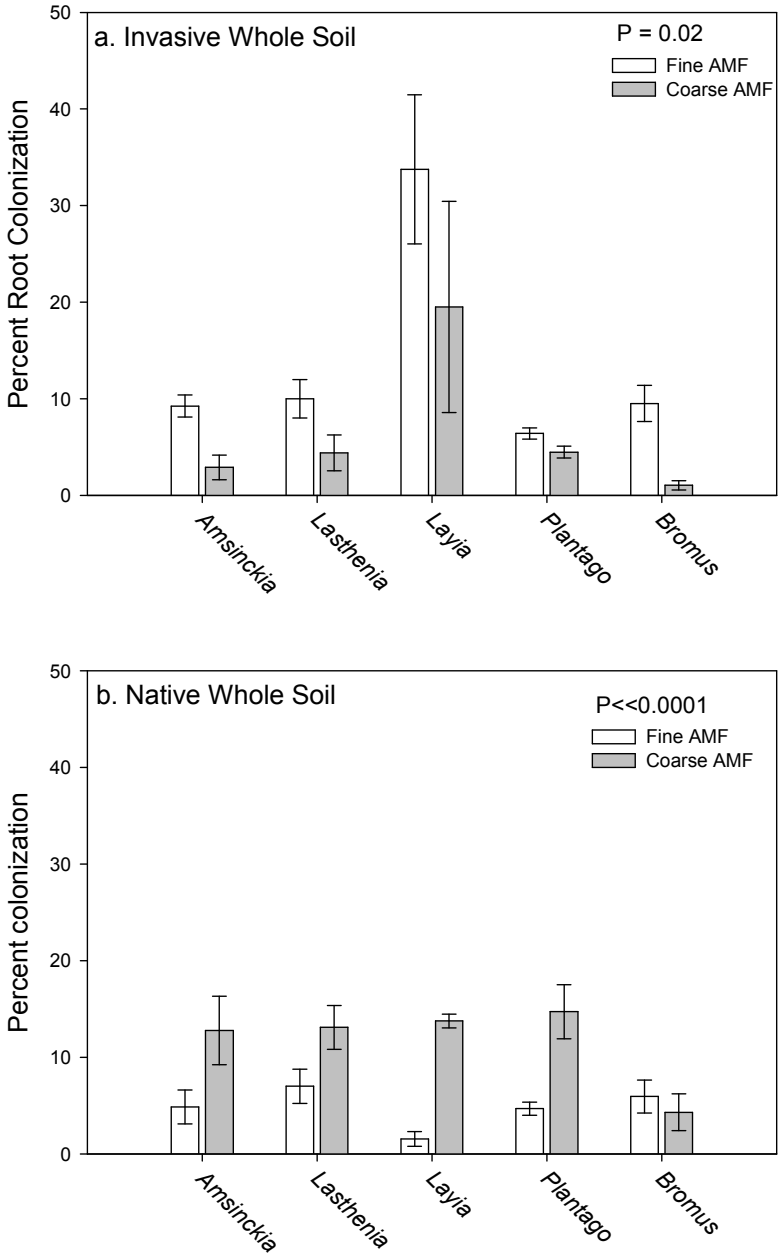

Figure 2.5 - Percent colonization of coarse and fine AMF in (a) invasive whole soil and (b) native whole soil for all five species. See Table 1 for percent colonization values for all species treatment combinations.

Table 2.1 - Percent Root colonization in all five species and seven soil treatments for plants grown in monoculture at week 6. Bold numbers denote values significantly different than the sterile treatment for each species and each microbial fraction at $P<0.05$

Treatment	Species	Fine AMF		Coarse AMF		Non-mycorrhizal Fungi		Oomycetes	
		Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Sterile	*Amsinckia menziesii*	5.0	4.1	0.0	0.0	1.5	1.2	0.0	0.0
	Lasthenia californica	0.3	0.3	0.0	0.0	1.5	1.5	0.0	0.0
	Layia platyglossa	8.3	3.0	0.0	0.0	2.3	0.6	7.4	3.2
	Plantago erecta	3.0	1.6	0.0	0.0	0.8	0.5	0.3	0.3
	Bromus diandrus	1.8	0.8	2.0	0.8	1.8	0.5	0.0	0.0
Native Whole Soil	*Amsinckia menziesii*	4.9	1.8	**12.8**	**3.5**	**13.9**	**1.5**	0.0	0.0
	Lasthenia californica	**7.0**	**1.8**	**13.1**	**2.3**	**22.7**	**4.0**	0.0	0.0
	Layia platyglossa	1.5	0.8	**13.8**	**0.7**	9.7	1.5	0.0	0.0
	Plantago erecta	4.7	0.7	**14.7**	**2.8**	**19.1**	**2.2**	0.0	0.0
	Bromus diandrus	5.9	1.7	4.3	1.9	**15.6**	**4.2**	0.0	0.0
Invasive Whole Soil	*Amsinckia menziesii*	9.2	1.1	2.9	1.3	**21.6**	**4.3**	0.0	0.0
	Lasthenia californica	**10.0**	**2.0**	**4.4**	**1.9**	**30.1**	**5.9**	0.0	0.0
	Layia platyglossa	**33.8**	**7.7**	**19.5**	**10.9**	12.0	3.5	**0.3**	**0.3**
	Plantago erecta	6.4	0.6	**4.5**	**0.6**	**26.2**	**4.1**	0.0	0.0
	Bromus diandrus	**9.5**	**1.9**	1.1	0.5	**15.5**	**6.3**	0.0	0.0
Native Filtrate	*Amsinckia menziesii*	1.1	0.8	0.0	0.0	7.5	2.0	0.0	0.0
	Lasthenia californica	3.5	2.3	0.3	0.3	5.9	1.0	0.0	0.0
	Layia platyglossa	4.4	1.7	0.0	0.0	9.6	1.9	0.0	0.0
	Plantago erecta	3.4	2.6	0.3	0.3	**8.6**	**1.6**	0.0	0.0
	Bromus diandrus	5.4	2.2	0.3	0.3	10.4	2.0	0.0	0.0
Invasive Filtrate	*Amsinckia menziesii*	4.1	0.5	1.3	0.8	**13.1**	**2.9**	0.0	0.0
	Lasthenia californica	3.0	0.6	0.0	0.0	**18.1**	**1.9**	0.0	0.0
	Layia platyglossa	**29.6**	**4.6**	0.0	0.0	16.2	7.8	0.0	0.0
	Plantago erecta	3.9	1.9	0.5	0.3	**13.6**	**2.9**	0.0	0.0
	Bromus diandrus	6.8	1.1	0.0	0.0	4.7	1.4	0.0	0.0
Native AMF Spores	*Amsinckia menziesii*	2.8	1.5	**9.9**	**2.2**	2.4	0.8	0.0	0.0
	Lasthenia californica	4.7	0.8	4.1	1.4	3.8	2.9	0.0	0.0
	Layia platyglossa	22.1	7.5	8.1	2.5	9.1	3.7	0.0	0.0
	Plantago erecta	**21.4**	**4.8**	**7.3**	**2.0**	4.2	0.7	0.0	0.0
	Bromus diandrus	4.0	0.5	2.7	0.9	1.8	1.0	0.0	0.0
Invasive AMF Spores	*Amsinckia menziesii*	4.8	1.0	3.6	1.0	5.3	1.3	**0.5**	**0.3**
	Lasthenia californica	**12.3**	**4.1**	**9.8**	**2.7**	4.8	1.1	**3.8**	**1.5**
	Layia platyglossa	**28.4**	**4.4**	2.1	0.8	14.9	4.5	3.9	3.6
	Plantago erecta	**9.8**	**2.7**	1.8	0.8	3.3	2.0	0.0	0.0
	Bromus diandrus	**9.6**	**2.9**	0.5	0.3	0.3	0.3	0.0	0.0

SOIL BIOTA IN ABANDONED AGRICULTURE LIMITS RESTORATION OF NATIVE FORBS IN SOUTHERN CALIFORNIA

ABSTRACT

Biotic and abiotic constraints on the re-establishment of native plant communities in abandoned agriculture may be due to strong soil legacy effects. Agricultural practices such as tillage, long-term fertilization, and cultivating monocultures can all contribute to soil degradation over time. Little is known about how soil-borne pathogen legacies affect the re-establishment of native plant species during restoration. I examined the effects of a fungicide (fludioxonil) treated soil and an oomycetecide (metalaxyl) treated soil compared to sterile soil and untreated control soil on the growth of the invasive annual grass *Bromus diandrus* and associated native forbs *Amsinckia menziesii*, *Layia platyglossa*, and *Lasthenia californica* growing in mixture. The application of fludioxonil increased aboveground biomass of the native forb *Layia platyglossa* (P=0.005) and the invasive grass *Bromus* (P=0.0001). *Bromus* had higher biomass in sterile soil than all other treatments, whereas *Layia* and *Lasthenia* had reduced biomass in sterile soils compared to control soils. Soil treatment had an effect on root colonization by coarse AMF and non-mycorrhizal fungi in all species. *Lasthenia* and *Layia* had decreased AMF colonization with metalaxyl. While other studies have demonstrated that metalaxyl increases AMF root colonization, I show that when metalaxyl is applied at the manufacture's recommendation it reduced AMF colonization compared to untreated controls. In contrast, fludioxonil decreased pathogens in *Lasthenia* while having no effect

on AMF colonization. *Bromus* also had increased biomass in soils treated with fludioxonil and sterile soils, suggesting its release from soil-borne pathogens with the application of fludioxonil. *Fusarium equiseti* and *Fusarium pseduograminerarum* were isolated from our soils and are known to cause root-rot and crown rot in wheat. They may negatively affect the growth of *Bromus* in this system, and the use of fungicides may benefit *Bromus* while benefitting some native forbs.

INTRODUCTION

The conversion of natural habitat to agriculture is one of the dominant forms of land degradation and loss of biodiversity throughout the world. The subsequent abandonment has led to some of the most dramatic plant invasions (MacDonald et al. 2000). In the United States alone, there are 68 million hectares of abandoned agricultural fields (Zumkehr and Campbell 2013). The process of returning these abandoned agricultural lands to native vegetation may require intensive management of soils, seedbanks, and vegetation (Marushia and Allen 2011). Previous studies of abandoned agricultural lands have increased our understanding of ecosystem recovery as well as successful restoration practices (Allen et al. 2005, Marushia and Allen 2011). However, these studies have focused on aboveground processes, and little is known about how the soil legacy of agricultural soils affects succession and restoration.

The soil microbial communities of agricultural lands are often less diverse than those of adjacent undisturbed soils (Steenwerth et al. 2002, McKinley et al. 2005, Kulmatiski and Beard 2008). Agricultural practices such as tillage, long-term fertilization, and monoculture cropping can all contribute to soil degradation over time (Cramer & Hobbs 2007). Old fields have been shown to contain low mycorrhizal inoculum potential (Miller 2002) as well as aggressive soil-borne pathogens (Mills and Bever 1998). Arbuscular mycorrhizal fungi (AMF) significantly influence plant growth and diversity (van der Heijden et al. 1998), and the outcome of restoration in old fields (Richter & Stutz 2002, Bever et al. 2003). Additionally, AMF often increase the ability of

plants to withstand pathogen infection (Borowicz 1981). Restoration of native prairie in abandoned agriculture with low mycorrhizal potential has had improved success by using mutualist facilitation (Miller 2002, Richter & Stutz 2002, Middleton and Bever 2012).

Local accumulation of soil-borne pathogens may help maintain plant diversity in natural systems (Petermann et al. 2008, Bever et al. 2012), while pathogens in agricultural lands may be less diverse but more aggressive in their impacts on crop plants (Altieri 1999). While it is believed that many crop pathogens are host-specific (Bullock 1992) and may not impact native plants, little is known about how pathogens in abandoned agriculture affect the re-establishment of native plant species and the success or failure of restoration in these systems. Agricultural soil pathogens may persist in the soil and cause different plant-soil feedback in native and invasive species during succession and restoration.

Few studies have examined the impacts of different management regimes and soil-borne pathogens on plant diversity. However, Bonanomi et al. (2013) found that *Agaricus compestris* affected the spatial distribution and diversity of co-existing plant species in abandoned grassland. Similarly, the local pathogen *Fusarium semitectum* was demonstrated to limit plant species distribution in the restoration of simulated slash and burn agriculture in a tropical dry forest (Allen et al. 2005). The effects of local pathogens on native and invasive species may vary significantly. For example, Hilbig and Allen (2015; Fig 4.1) found oomycete pathogenic hyphae infecting the roots of native coastal sage scrub forb species but not the invasive grass *Bromus diandrus* when plants were inoculated with abandoned agricultural soils conditioned by *Bromus diandrus*.

Oomycetes of the genera *Phytophthora* and *Pythium* are responsible for many noxious pre- and post-harvest crop diseases including citrus brown rot (Cohen and Coffey 1986).

Control of soil-borne pathogens through the application of general fungicides is a common agricultural practice but seldom done in restoration. The phenylpyrrole fludioxonil is a non-systemic, broad-spectrum fungicide with long residual activity (Rosslenbroich and Stuebler 2000). Through the inhibition of glycerol biosynthesis, fludioxonil inhibits spore germination, germ tube elongation and mycelium growth (Rosslenbroich and Stuebler 2000). Fludioxonil is commonly used in seed and post-harvest treatments of citrus fruits (Zhang 2007). It effectively controls many noxious agricultural pests of the genera *Penicillium*, *Fusarium*, and *Lasiodiplodia* (Zhang 2007). The phenylamide metalaxyl is an oomycete fungicide, or oomyceticide, that is used to protect over 100 agricultural crops through the control of *Pythium* and *Phytophthora* species (Fisher and Hayes 1982, Davidse et al. 1988). Through the inhibition on uridine incorporation in RNA polymerase-1, metalaxyl inhibits mycelial growth in fungi (Sukul and Spiteller 2000). In addition to controlling oomycete pests, metalaxyl has been shown to stimulate AMF colonization when applied at low levels (Afek et al. 1991, and Hetrick and Wilson 1991).

The overall objective of our study was to examine the role of soil biota in the restoration of abandoned citrus agriculture invaded by a Mediterranean annual grass, *Bromus diandrus*. I manipulated soil fungal communities in field and greenhouse studies through the use of fludioxonil and metalaxyl to determine if the suppression of different

fungal functional groups present in abandoned agriculture compared to sterile soil and an untreated control affects native forb and invasive grass establishment and growth.

MATERIAL AND METHODS

Study Site

The study site was at the Lake Mathews Riverside County Habitat Conservation Agency (RCHCA) lands located in western Riverside County, California (33.36°N, 117.02°W). The area has a Mediterranean-type climate that receives an average of 262mm precipitation, mostly during the winter-spring growing season (November to May). During the year of the experiment, 2012-13, the precipitation was only 118mm. The reserve is approximately 20, 200 ha of historically disturbed coastal sage scrub and annual forbland (Minnich 2008). Much of the land was converted to a citrus orchard beginning in the early 1900s. Citrus agriculture at Lake Mathews was abandoned approximately 25 years ago when irrigation ceased, but restoration planning and permitting did not begin until 2004. Removal of citrus trees and agricultural infrastructure started 2006 and is still underway. Since abandonment the land has been heavily invaded by the exotic annual grass *Bromus diandrus*, and has transitioned to exotic annual grassland.

Field experiment

In September 2012, fifteen 2m x 2m experimental plots were established in five replicated blocks with three soil treatments: (1) fludioxonil, (2) native coastal sage scrub inoculum and (3) inoculum from a citrus orchard where trees had been cut and removed nine years previously. Plots were established in an area that contains remnant citrus tree stumps (avoiding stumps) to test the effects of fludioxonil and native inoculum on native forb and *Bromus diandrus* establishment. Fungal mortality due to the use of fungicides can result in a flush of nutrients; to control for changes in soil nutrient concentrations associated with the application of fungicides all plots were treated with CANNONBALL WP (Syngenta, Greensboro NC) at the manufacturer's recommended concentration and later inoculated. Fludioxonil is the active ingredient in CANNONBALL WP. Fludioxonil plots received five fungicide applications throughout the growing season. All other plots received a thin layer of soil inoculum collected from (1) rhizosphere of adjacent native coastal sage scrub or (2) abandoned citrus agricultural lands. Inoculum soil was collected to a depth of 5cm in shovelfuls randomized across an area of about 1 ha in each of the two inoculum source areas. Approximately 425 liters of soil were collected and homogenized from each area. Fresh inoculum was added to a depth of 4cm in five lines of 6cm width every 0.5m of the plot three weeks after fludioxonil was applied, per manufacture's recommendation to reduce residual effects of the fungicide on live inoculum. Trenches were also dug in fludioxonil plots without inoculum to control for the disturbance.

Plots were seeded with a mixture of native forb species at 400 viable seeds/m^2 at the same time soil inoculum was added. Seed mix included equal quantities of *Amsinckia menziesii, Lasthenia californica*, *Layia platyglossa*, *Plantago erecta*, and *Hemizonia fasciculatum*. These were chosen because they are among the most abundant native annuals based on a 2010 vegetation survey completed at Lake Mathews (Allen unpublished data). Plots were sampled at peak flowering time for plant species richness, density, percent cover, and above-ground biomass. Data were collected on the entire 2 x 2 m. Early rains in November 2012 promoted germination from the seedbank and of seeded annuals, but due to severe mid-season drought most of the annuals did not survive to flowering or seed production and the experiment was terminated in January, 2013. The next growing season, 2013-14, had equally low precipitation, 140mm, and even lower emergence and survival of winter annuals, so the experiment was moved into the greenhouse.

Greenhouse Experiment

In February 2014, native forb species associated with coastal sage scrub and the invasive grass *Bromus diandrus* were seeded in 4-gallon pots (38.7cm x 29.2cm x 18.4cm) and grown in a controlled greenhouse condition for five weeks after emergence. The native forbs were *Amsinckia menziesii*, *Lasthenia californica*, and *Layia platyglossa.* Pots were arranged in a randomized complete block design to control for potential temperature gradients in the greenhouse.

Soil was collected at Lake Mathews (Potterville cobbly clay, Nelson et al. 1919) from sparsely vegetated soil (due to the drought) that was previously a stand of *Bromus diandrus*. The field experiment above and a prior greenhouse experiment (Hilbig and Allen 2015) showed few to no significant effects of native inoculum, so only invasive inoculum was tested in this study. Soil was cut fifty percent with silica sand to create a sandy clay for better drainage; this is a common practice for inoculum studies in fine-textured soil (e.g. Johnson et al. 2008, Hilbig and Allen 2015). All soil was steam-sterilized for 24 hours, held at room temperature for 24 hours, and sterilized for another 24 hours. Four soil treatments were created: sterile, control, metalaxyl-treated soils, and fludioxonil-treated soils with 5 replications per treatment. Sterile pots were left without live inoculum, all other treatments received 4cm of live inoculum cut fifty percent with sterilized sand to the top of pots. Live inoculum was the original soil collected from Lake Mathews and also cut fifty percent with silica sand. CANNONOBALL WP was immediately applied to pots with the fludioxonil soil treatment and throughout the experiment at the manufacture's recommendations. Similarly, metalaxyl-treated pots received Subdue (Syngenta, Greensboro NC) at the manufacture's recommendations. Previous greenhouse trials included sterile soils with metalaxyl and fludioxonil applications. Plant biomass did not differ significantly between sterile soil and sterile soils with either fungicide ($P>0.05$) demonstrating that metalaxyl and fludioxonil did not have any direct toxicity to plants.

Prior to the start of the experiment, pots were watered for two weeks to promote germination of seeds that might have been viable and present in the live inoculum.

Seedlings were removed and seeds of the three native forbs and *Bromus diandrus* were added at a density of 6 viable seeds per species per pot. *Amsinckia* germinates a week after other species and was therefore seeded a week before *Lasthenia, Layia,* and *Bromus*. Pots contained a constant density of 24 plants across all treatments with six individuals of each species.

Plants were harvested after five weeks for aboveground biomass and root biomass. Biomass was determined after drying at 60℃ for 48 hours. Total root biomass was collected per pot, as it is too difficult to untangle the fine roots of every individual within the pot. Roots were washed of soil, and biomass was determined after drying at 60℃ for 48 hours. Some fine roots for species were collected to determine percent root colonization as described below. These roots could be identified to species because they were attached to stems, and they were dried, weighed and added to the total biomass per pot.

Fungal Root Colonization

Roots were examined for structures of both AMF and non-mycorrhizal fungi of Ascomycota and Basidiomycota as described by Hilbig and Allen (2015). Dried roots were rehydrated, cleared in 2.5% KOH, acidified in 1% HCl, and stained with 0.05% trypan blue (Kormanik and McGraw 1982, Koske and Gemma 1989). Previous studies observed that drying did not change percent colonization of mycorrhizal or potential pathogenic fungi (Hilbig and Allen 2015). Root fragments were then mounted in PVLG

on slides, and eighty observations at 400X were made for the root system of each individual (n=480).

Culturing and Identifying Pathogens

Soil cores were collected at Lake Mathews underneath remnant citrus stumps. Fifteen grams of soil were then mixed with 50 mL of sterile DI H_2O. Suspension was allowed to passively settle for an hour before supernatant was pipetted onto PARP-H plates. Plates were left in the dark at room temperature for 2 days, after which supernant and soil were washed from plates with sterile DIH_2O. Rinsed plates were then left to incubate in the dark at room temperature for 7 days.

Fungal DNA was extracted from mycelium using the DNeasy Plant Mini Kit per manufacture's protocol (Qiagen, Valencia, CA). To assess the purity of extracted DNA the ratio of absorbance at 260nm and 280nm using the Thermo Scientific NanoDrop™ 1000 Spectrophotometer was assessed for each sample. The general purpose fungal primers ITS 4 5'TCCTCCGCTTATTGATATGC and ITS 5 5'GGAAGTAAAAGTCGTAACAAGG were used to amplify the internal transcribed spacer region (ITS), which includes ITS1, 5.8S, and the ITS2 regions.

Each PCR mixture (50µl) contained 3µl DNA, 27.6µl distilled deionized H_2O, 5µl $MgCl_2$ [25mM], 10µl 5XMg free buffer, 4µl 2.5mM dNTP, 0.1µl of ITS 4 primer [50µM], 0.1µl of ITS 5 primer [50µM], and 0.2µ of GoTaq. Amplification was conducted in a thermal cycler under the following conditions: 40 cycles of 92°C for 2 min, 55°C for 2 min, and 72°C for 2.5 min, and a final extension of 10 min at 72°C

(White et al., PCR Protocols, Academic Press, 1990). A negative control of no template DNA was included in each PCR reaction. PCR products were analyzed by electrophoresis on 2% agrose gel on 1XTBE and visualized by ethidium bromide staining. The PCR products were purified using the single step enzymatic cleanup product ExoSAP-IT® following the manufacturer's protocol. Amplification primers were also used for sequencing fragments in both directions. Sequencing was done at the University of California Riverside Genomic Core Facilities. The ITS sequences were compared with known sequences in the GeneBank database (www.ncbi.nlm.nih.gov.BLAST).

Statistical Analyses

For field plots, plant density and percent cover for native and invasive species were analyzed using separate one-way ANOVA for each species, and for native and invasive species grouped with soil treatment as a fixed factor. For the greenhouse study, aboveground biomass was analyzed using separate one-way ANOVA for each species with soil treatment as a fixed factor. Below ground biomass was grouped per pot as total roots at five weeks as they could not be separated by species. Therefore, root biomass was analyzed across pots using a one-way ANOVA with soil treatment as a fixed factor. Soil treatments were compared using least significant difference ($L.S.D._{.0.05}$). All data were checked for homogeneity of variances using Levene's tests, and for normality using the Shapiro-Wilk test. Percent root colonization data failed to meet the normality assumption even after a log transformation, and were analyzed using Kruskal-Wallis rank sum test for each species with soil treatment as a fixed factor. Soil treatments were

compared using Nemenyi's test. Box and whisker plots were used to detect outliers, and one metalaxyl pot was removed prior to statistical analyses because of a high biomass >180% of the mean. All statistical analyses were performed using R version 3.0.2 (R Development Core Team 2013).

RESULTS

Field Experiment

Excessive drought in the 2012-13 growing season coupled with warm temperatures resulted in approximately 82% bare soil. The only native forb to establish from the seed mix and possibly the soil seed bank was *Amsinckia menziesii*. The average density of *Amsinckia menziesii* in control plots was only 1.8/m^2, 21.1/m^2 in plots with fungicide, and 0/m^2 in plots with added native inoculum (data not shown). There were no significant differences between plots with added inoculum and control plots, perhaps due to some high within treatment variation. Five non-native, invasive species established from the soil seed bank including *Avena fatua*, *Bromus diandrus*, *Hirschfeldia incana*, *Erodium cicutarium*, and *Salsola tragus*. However drought resulted in low establishment of invasive species, and there was no significant soil treatment effect on their density or cover (data not shown).

Greenhouse Study

Total native forb aboveground biomass varied across soil treatments (P=0.016). Pots treated with fludioxonil had greater native forb biomass than those with sterile soil or soil treated with metalaxyl. The application of fludioxonil increased aboveground biomass of *Layia* (P=0.005; Fig 4.2a) and *Lasthenia* (P=0.020; Fig 4.3a), but had no effect on *Amsinckia* biomass (Fig 4.4a). *Bromus* aboveground biomass varied across all soil treatments (P<<0.001, Fig 4.5a). It was greatest in sterile soil and lowest in soils treated with metalaxyl (P<<0.001, Fig 4.6a). Root biomass was not significantly different across soil treatments, and there was some high within-treatment variation (Fig 4.6).

Fungal Root Colonization

Roots of native and invasive species were colonized by both fine and coarse AMF, and non-mycorrhizal fungi. AMF colonization was reduced in *Lasthenia* when soils were treated with metalaxyl compared to the control (P<<0.001 Fig 4.3b). Fludioxonil reduced non-mycorrhizal colonization compared to metalaxyl in *Lasthenia* (P<<0.001 Fig 4.3b). *Layia* showed similar trends of decreased AMF colonization with the application of metalaxyl (Fig 4.2b). *Amsinckia* had the lowest AMF colonization of the native forbs (Fig 4.4b) and there was no significant effect of either pesticide on AMF and non-mycorrhizal colonization. Similarly, there was no significant effect of either pesticide on AMF and non-mycorrhizal fungi in *Bromus*, but sterile soil significantly reduced coarse AMF and non-mycorrhizal fungi (P=0.004 and P=0.03 respectively).

Cultured Pathogens

Culturing and subsequent isolation of potential oomycetes using PARP-H media resulted in 5 morphologically unique cultures. Sequences of our cultures matched known sequences of one oomycete (*Pythium heterothallicum*), three ascomycetous fungi (*Fusarium equiseti, Fusarium pseudograminerarum, Penicillium brevicompactum*), and one zygomycete (*Mortierellales sp*) within GeneBank.

DISCUSSION

Both native and invasive species performed poorly when soils were treated with metalaxyl. Pesticides can have both negative and positive effects on mycorrhizal fungi due to differences in a fungicides' mode of action, chemical structure, release of soil mineral nutrients, and the method of application (Johnson and Pfleger 1992, Jin et al. 2013). Release of soil nutrients with ascomycete mortality due to fludioxonil could result in increased biomass of plants. Our soils were steam sterilized across all treatments and only 4cm of live inoculum cut fifty percent with sterilized sand was added to pots, therefore any potential flush of nutrients would have been small. Additionally, I would expect an increase in biomass in fludioxonil compared to sterile soils to occur in all species as all species received the same soil inoculum. *Amsinckia* and *Bromus* did not have higher aboveground biomass in fludioxonil treated soils than sterile. This suggest that the changes in biomass among soil treatments is due to differences in the soil biota.

There were few effects of metalxyl on total non-mycorrhizal colonization, so it is likely that the observed negative effects of metalaxyl on growth might better be explained

by non-target effects on AMF. The reported effects of metalaxyl on AMF are highly variable. Most studies have demonstrated that low levels of metalaxyl increase AMF colonization (Groth and Martison 1983, Afex et al. 1991, and Hetrick and Wilson 1991, Johnson and Pfleger 1992, Shetty and Magu 1997). The beneficial effects of metalaxyl on mycorrhizal colonization could be due to the suppression of organisms that are antagonistic towards AMF (Johnson and Pfleger 1992). Alternatively, studies have found that metalaxyl restricts AMF when applied as soil drenches (Jabaji-Hare and Kendrick 1987, Carrenho et al. 2000). Jabaji-Hare and Kendrick (1987) found that soil drench applications of metalaxyl, Ridomil (Syngenta, Greensboro NC), reduced mycorrhizal colonization in leeks at 0.5, 1.0, and 2.0 mg active ingredient per plant. Similarly, Oliveira (1992) found low levels of mycorrhizal colonization in Rangpur lime when metalaxyl was applied at 1.0 mg ml^{-1} and 2.0mg ml^{-1}.

The mechanisms behind suppression or enhancement of mycorrhizal colonization with applications of metalaxyl are poorly understood (Johnson and Pfleger 1992). It has been suggested that response to oomyceticide is AMF species specific, and specific to host plant species (Jabaji-Hare and Kendrick 1987). This body of research is done entirely on crop species. It is possible that responses to metalaxyl in native plants of natural systems would vary from an agricultural system. Additionally, the chemical used, the rates of application, and method of application account for the observed different AMF responses to metalaxyl. I applied Subdue through soil drenching applications at the manufacture's recommendation, 1.9 mg active ingredient per plant, throughout the experiment. Due to the application method, the benefits of oomycete pathogen protection

from metalaxyl are offset by its detrimental effects on AMF in these highly mycorrhizal native forbs as seen in *Lasthenia and Layia*. Soil drench applications of high concentrations of metalaxyl could have resulted in the accumulation of the oomyceticide within the root zone of soils. This systemic oomyceticide could then be taken up by plant roots at the site and timing of AMF colonization. *Amsinckia* had lower AMF colonization than other native forbs in control soils, and the reduction in AMF seen in the other native forbs was not found in *Amsinckia*. *Amsinckia* might rely less on AMF in this system than other native forbs and, therefore was not negatively impacted by the application of metalaxyl.

While metalaxyl resulted in decreased plant growth, the application of fludioxonil increased biomass in *Bromus*, *Lasthenia* and *Layia*. Increased biomass with fludioxonil application is most likely due to a reduction in infection by soil-born root pathogens while having no negative effect on coarse AMF, so that the ratio of AMF to non-mycorrhizal fungi is increased as seen in *Lasthenia* and *Layia*. Fludioxonil has been shown to drastically increase root colonization by AMF (Murillo-Williams and Pedersen 2008) while being effective against pathogens, such as *Fusarium* and *Penicillium* species. AMF interact in the rhizosphere and compete with co-occurring pathogens for the same colonization sites (Smith and Read 2008). Increases in AMF colonization with fludioxonil may be due to reduced competition with aggressive pathogens for those colonization sites (Murillo-Williams and Pedersen 2008).

The use of fludioxonil during restoration of abandoned agriculture might result in increased establishment and growth of native forb species. However, *Bromus* had

increased biomass in soils treated with fludioxonil and sterile soils, suggesting its release from soil-borne pathogens with the application of fludioxonil. Root colonization by non-mycorrhizal fungi was not significantly reduced in *Bromus* with fludioxonil. This could be due to some pathogenic or saprophytic fungi that are not targeted by fludioxonil. Increased biomass suggested that fludioxonil might decrease the colonization by aggressive pathogens while having no effect on other non-mycorrhizal fungi.

I cultured and identified five agricultural pathogens including *Fusarium equiseti* and *F. pseduograminerarum* that may have been reduced by fludioxonil. These pathogens are known to cause root-rot and crown rot in wheat, respectively (Wilcoxson et al. 1988). While there is no reported history of wheat being grown at Lake Mathews, agricultural pests are easily transported between fields through equipment. Weed and native plant species have been demonstrated to serve as alternate reservoir hosts for pathogens, or obligate alternate host for pathogens such as rusts (Gail and Robert 2005, Wisler and Norris 2005, Burdon and Thrall 2008). In this system, *Fusarium equiseti* and *F. pseduograminerarum* may use *Bromus* as an alternate host to wheat and negatively affect *Bromus* growth without causing mortality. Other *Fusarium* species have been found to increase seed mortality in *Bromus tectorum* (Meyer et al. 2014). Soil legacies of cultivated lands may affect the establishment of both native and invasive plant species during restoration. Applications of fungicides, such as fludioxonil, might actually benefit the already competitively superior invasive species at the same time it benefits some native species. Fludioxonil may be beneficial in restoration only when invasive species have no naturalized enemies.

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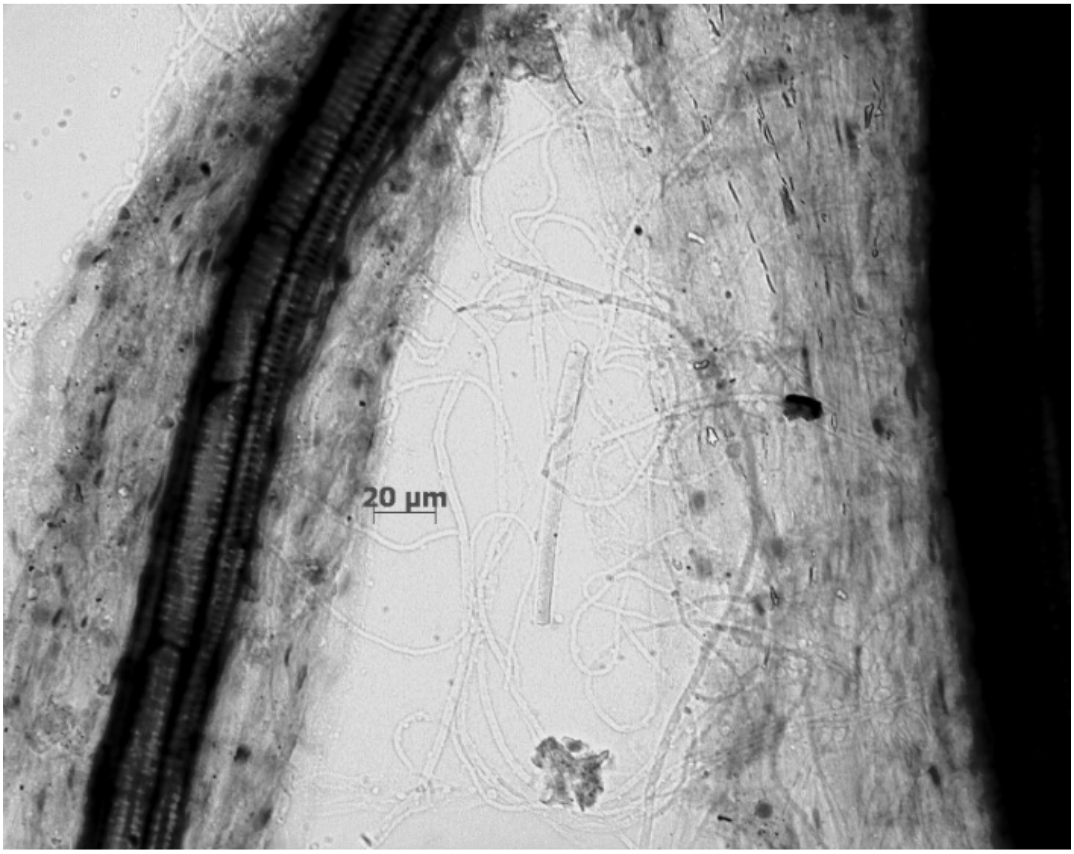

Figure 4.1 - Image of native forb *Layia platyglossa* roots infected with aseptate oomycete hyphae. Observation was made at 400X magnification.

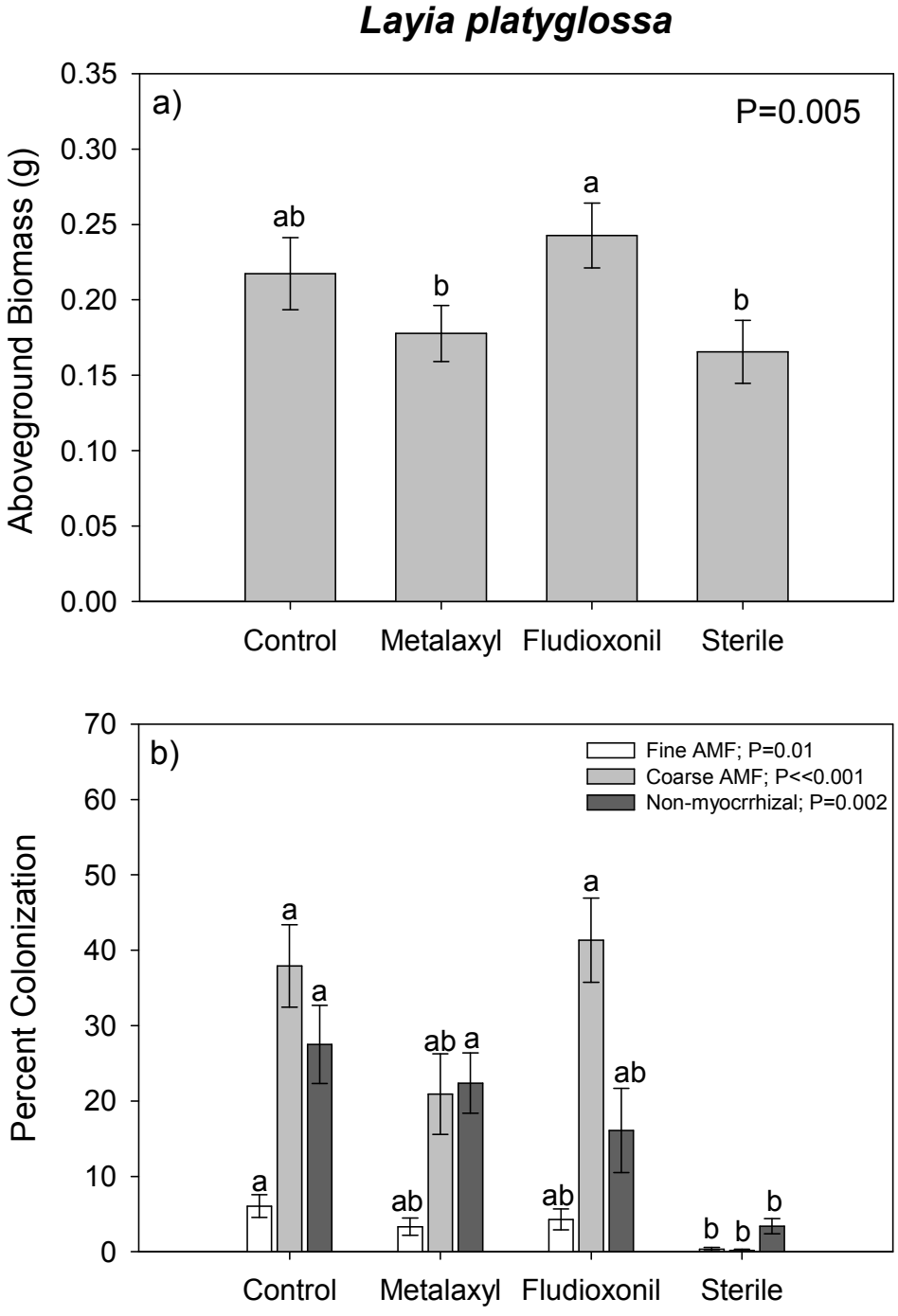

Figure 4.2 - a) Aboveground biomass and b) percent root colonization of fungi in *Layia platyglossa* at week 5. Separate Kruskal-Wallis tests were run for each fungal group with treatment as a fixed factor. Significance was determined at $\alpha=0.05$.

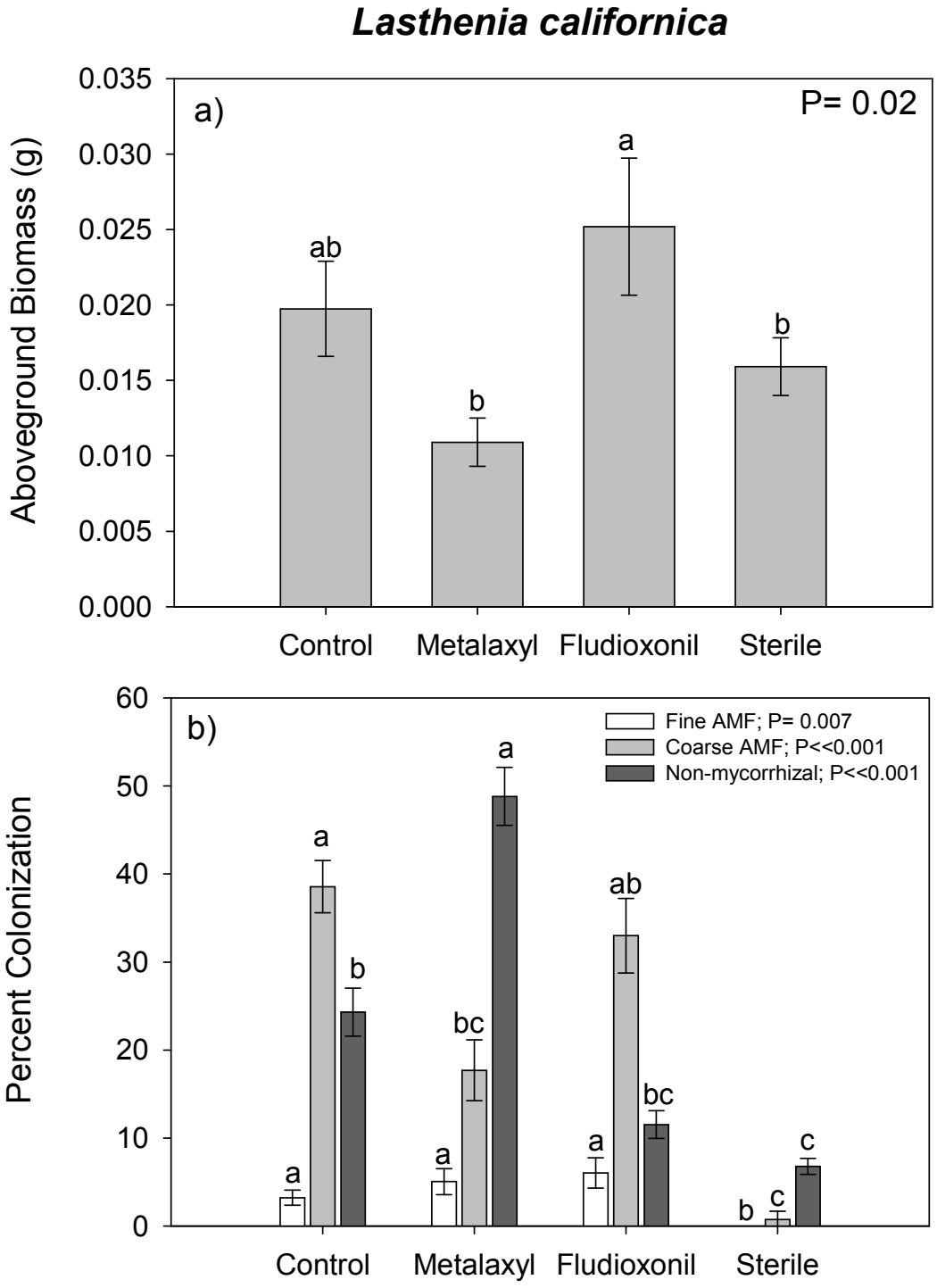

Figure 4.3 - a) Aboveground biomass and b) percent root colonization of fungi in *Lasthenia californica* at week 5. Separate Kruskal-Wallis tests were run for each fungal group with treatment as a fixed factor. Significance was determined at $\alpha=0.05$.

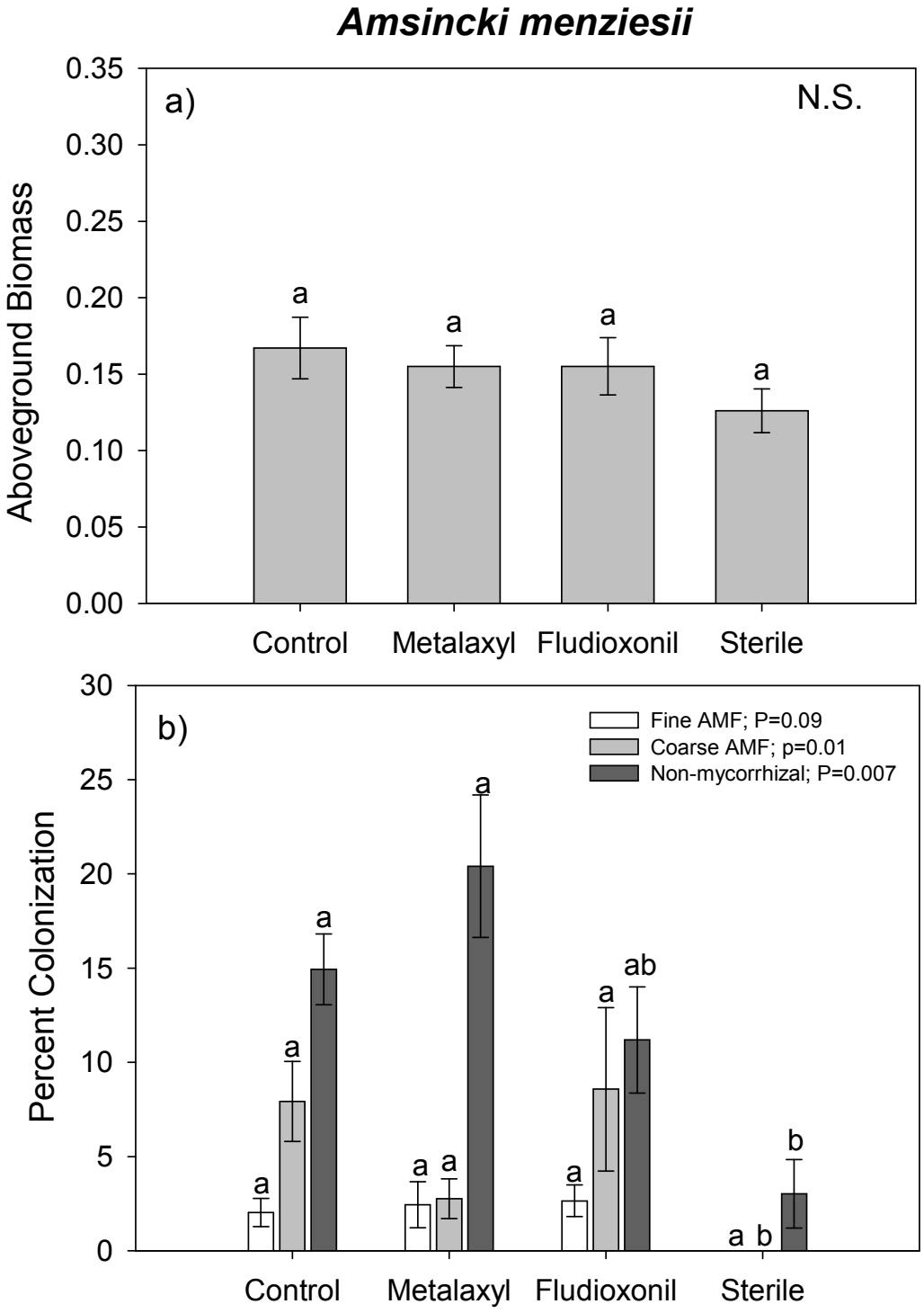

Figure 4.4 - a) Aboveground biomass and b) percent root colonization of fungi in *Amsinckia menziesii* at week 5. Separate Kruskal-Wallis tests were run for each fungal group with treatment as a fixed factor. Significance was determined at α=0.05.

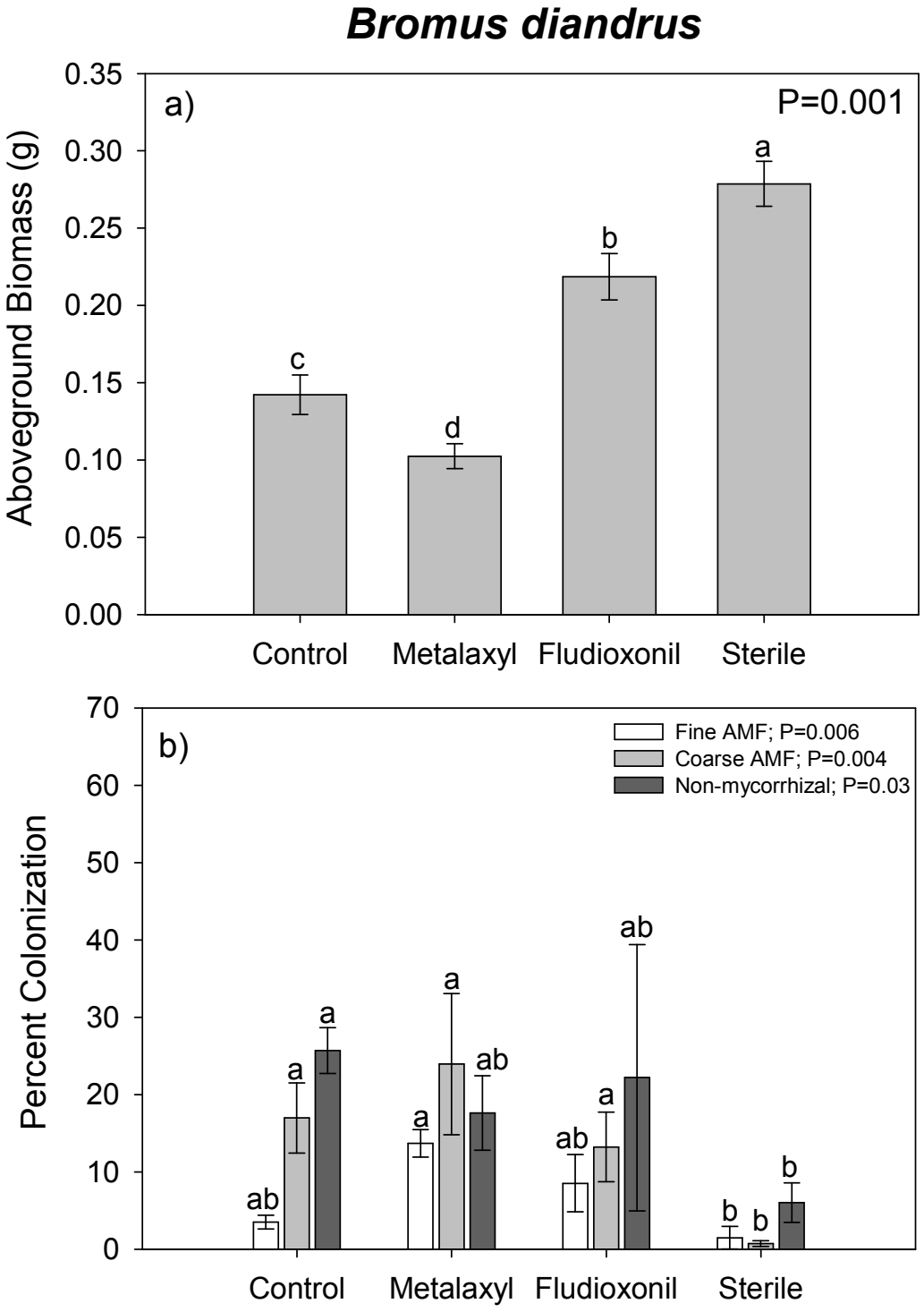

Figure 4.5 - a) Aboveground biomass and b) percent root colonization of fungi in *Bromus diandrus* at week 5. Separate Kruskal-Wallis tests were run for each fungal group with treatment as a fixed factor. Significance was determined at $\alpha=0.05$.

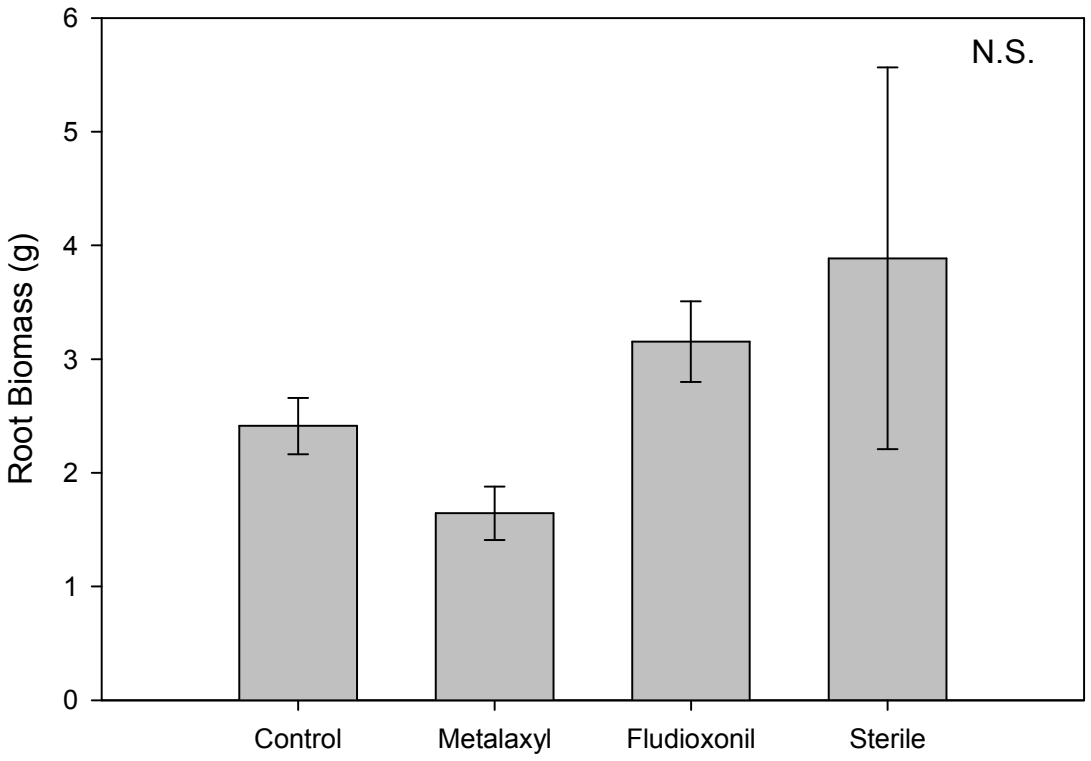

Figure 4.6 - Root biomass of all species per pot (native forbs and *Bromus diandrus*) when grown under different soil treatments. Significance was determined at α=0.05

GENERAL CONCLUSIONS

California coastal sage scrub (CSS) and associated forblands have been historically disturbed by agriculture, fire, grazing, and fragmentation (Minnich and Dezzani 1998, Minnich 2008). These disturbances have promoted the establishment of exotic annual grasses, and once established, exotic annual grasses persist (Allen *et al.* 2000). The Mediterranean annual grass *Bromus diandrus* is among one of the dominant invasive species in CSS communities (Barbour et al. 2007). The establishment and spread of *B. diandrus* may be attributed to a complex of multiple mechanisms. Understanding the relative importance of mechanisms contributing to the success of *B. diandrus* can inform successful restoration of invaded ecosystems. The studies of this dissertation examined morphological traits relative to traits of native species, plant-soil feedback, and soil legacy effects as mechanisms of *B. diandrus* invasion.

Synthesis of empirical data

Direct competition from *Bromus diandrus* may limit the coexistence between functionally similar species when fitness inequalities between *B. diandrus* and native species result in a competitively superior *B. diandrus* (MacArthur and Levins 1967, Keddy and Weiher 1999, Funk et al. 2008, Gotzenberger et al. 2012). Functionally similar native plant communities did not demonstrate biotic resistance to *B. diandrus* invasion during restoration studies. Other studies have also demonstrated that invasive

monocot species are not limited by functionally similar native species (Cahill *et al*. 2008, Price and Pärtel 2013). *B. diandrus* and *Amsinckia menziesii* are similar in many measured morphological traits related to the competitive ability of a plant (i.e. height and biomass) and both flower at the same time of year, but *A. menziesii* was a poor competitor with *B. diandrus* despite *A. menziesii* having a larger relative growth rate. Phenology differences in native and invasive species give an invasive species an advantage when they promote stabilizing niche differences that then make coexistence between these phenologically offset competitors more difficult (Godoy and Levine 2014). Earlier germination in *B. diandrus* results in offset competition that negatively impacts those species germinating later.

The soil microbial community acts as a driver of the plant community (Grime et al. 1987, van der Heijden et al. 1998, Klironomos 2002). Positive plant-soil feedback is a second mechanism contributing to the success of *B. diandrus* in CSS communities. Upon establishment *B. diandrus* shifts the arbuscular mycorrhizal fungi (AMF) community from large-spored, coarse AMF to small-spored, fine AMF or *Glomus tenue*. Native plants were infected more heavily with coarse AMF when grown with native inoculum whereas *B. diandrus* was always colonized more by fine AMF. Fine AMF appeared to cause most of the observed and positive feedback effect in *B. diandrus* even with the presence of pathogenic fungi in roots, suggesting fine AMF are more important to plant growth than pathogens during *B. diandrus* invasion. Native species in monoculture experienced an unexpected neutral to positive feedback with whole soil and filtrate inoculum that would contain potential pathogenic fungi. This suggests that there is not a

buildup of host-specific pathogens in native soils that would limit native forb establishment. Furthermore, calculated feedback changed in magnitude in the context of competition and, therefore highlights the difficulty of extending feedback results in monoculture to the community level.

Legacy effects to abiotic and biotic properties of soils can affect plant community assembly (Kardol et al. 2007, van der Voorde et al. 2011). Agricultural pests that remain in the soil beyond cultivation practices can cause disease in native and naturalized plants. The legacy of soil pathogens in our system reduces plant growth in all species except the native forb *A. menziesii*. Two agricultural pathogens isolated from the soil, *Fusarium equiseti* and *F. pseduograminerarum*, cause reduced biomass in *B. diandrus*. The use of the fungicide fludioxonil resulted in increased biomass in both native and invasive species. Fludioxonil appeared to reduce colonization of potential pathogenic fungi in native forbs *Layia platyglossa* and *Lasthenia californica*. In contrast, the oomyceticide metalaxyl appeared to have non-target effects on AMF, while having little effect on potential pathogens.

Implications

Many potential mechanisms of invasion have been proposed and tested over the past decades (Keane and Crawley 2002, Klironomos 2002, Callaway and Ridenour 2004, Vogelsang and Bever 2009). These theories of invasion are not mutually exclusive, and understanding biological invasions will rely on our ability to integrate them (Shea and Chesson 2002). The studies in this dissertation show that multiple mechanisms of

invasion promote *Bromus diandrus* success during attempted restoration of abandoned agriculture. The earlier germination and larger seed mass of *B. diandrus* allows this invasive grass to establish even in the presence of morphologically similar native species with greater relative growth rates. This suggests that timing of germination is more important than morphological traits in annual grass invasion, and successful restoration of invaded annual grasslands should rely on manipulating germination cues.

Positive plant-soil feedback in *B. diandrus* contributes to its overall success. The fine AMF has received little attention but may be very important in plant invasion. Native species are more frequently colonized by coarse AMF when both fine and coarse AMF are present. Both native and invasive inoculum contain fine AMF and, therefore mycorrhizal inoculum from native CSS would not facilitate native forb establishment during restoration of a *B. diandrus* invaded community.

Strong soil legacies in abandoned agriculture also contribute to *B. diandrus* invasion and inhibit successful reestablishment of native plants. Root fungal pathogens found in abandoned agricultural fields result in decreased biomass of some native species as well as *B. diandrus*. General fungicides such as fludioxonil can result in increased biomass through the release of soil borne pathogens. However, they benefit the already competitively superior invasive species at the same time they benefit some native species.

Future directions

During restoration, seeding with functionally similar natives while manipulating germination cues, combined with facilitated microbial inoculations might reduce *Bromus*

diandrus establishment. However, many mechanisms contribute to the overall success of this invasive species making it competitively superior, and eradication of *B. diandrus* on a large scale is unlikely. Current large-scale efforts at the Lake Mathews field site include grazing and fire, but these require continuous effort to maintain reduced densities of *B. diandrus*. Biological control agents that specifically target *Bromus diandrus* will greatly increase the possibility of restoration. Smut disease in *Bromus* species caused by the fungal pathogen *Ustilago bullata* is a one potential biocontrol organism. *U. bullata* is a seedling-infecting pathogen that grows systemically and sporulates in the host inflorescence eliminating seed production (Meyer *et al.* 2010). This potential biological control might result in greater restoration success and requires more consideration in our efforts to eradicate *B. diandrus*.

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