UNIVERSITY OF CALIFORNIA,
IRVINE

Ecological restoration of fungi

DISSERTATION

submitted in partial satisfaction of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

in Ecology and Evolutionary Biology

by

Mia Rose Maltz

Dissertation Committee:
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DEDICATION

To my family, who have supported my decisions, my education, and my entire life.
To my parents, Rhona and Harvey Maltz, I dedicate this body of research to you.
Thank you for being my greatest advocates and for encouraging me to explore the facets of mycology that I found most fascinating. I am grateful for your kindness, patience and empathy as I completed my dissertation research and struggled along this academic path.

To my loving husband Matthew Garrambone, who gave me the courage, insight, and motivation to achieve my goals. Thank you for co-parenting with me and for your unyielding support as I attained this doctoral degree. I look forward to returning the favor.
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I am grateful for my current and former colleagues in the Microbial Group and the Ecology Group of the Department of Ecology and Evolutionary Biology. It has been a great learning experience and an honor to be among this fine community of scholars.

My research on ecological restoration of fungi is motivated by my desire to explore techniques for rehabilitating damaged lands, preserving biodiversity, and conserving wild places.
My sincere hope is that my research can contribute to protecting our natural resources for our children, future generations, and all inhabitants of the ecosystem.

Finally, I offer my gratitude to the mushrooms.
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ABSTRACT OF THE DISSERTATION

Ecological restoration of fungi

By

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Doctor of Philosophy in Ecology and Evolutionary Biology

University of California, Irvine, 2016

Professor Kathleen K. Treseder, Chair

Ecosystem degradation may reduce biodiversity, including plants and fungi. Although restoration ecologists rarely specify fungal restoration as a desired outcome, restoration methods may either promote or inhibit fungi. Direct fungal restoration may provide benefits to ecosystems and support restoration efforts, but little is known about how either degradation or restoration affect fungi.

We investigated how fungi respond to habitat fragmentation in coastal sage scrub (CSS) ecosystems. Using molecular and fluorimetric techniques, we found that leaf-litter fungal activity declined in smaller fragments with less diverse vegetation. We manipulated plant litter diversity using litter bags and found less mass remaining in litter bags containing more diverse litter. Moreover, these litter bags harbored greater numbers of fungal taxa. Our findings suggest that plant diversity controls decomposer fungal diversity and function in fragmented ecosystems.

Fragmented landscapes are particularly vulnerable to plant invasions. Both invasive plants and invasive removal methods may harm native plants and fungi. Within invaded CSS ecosystems, we compared invasive plant removal methods, monitored plant communities, and extracted soil fungi. While both herbicide and mowing reduced invasive cover, neither method

promoted native plant recovery. Herbicide however had the added benefit of facilitating fungal recovery.

Mycorrhizal fungal recovery may support restoration outcomes by providing benefits to plants. However, it is unclear whether directly manipulating mycorrhizal fungi via inoculation has consistent effects across ecosystems or long-term effects on colonization, and whether types of inocula differ in their effectiveness. We synthesized data from restoration studies across ecosystems to examine the effects of inoculation on mycorrhizal establishment and plant growth under field conditions. We found that inoculation consistently increased mycorrhizal abundance across ecosystem types and improved growth and establishment of plants long-term. Both fungi from reference ecosystems and species-specific inoculum improved mycorrhizal associations with plants more than did commercial fungi.

Landscape degradation inhibits certain groups of fungi that either cycle nutrients or support plants. Depending on the method used, restoration may either not have any effect on fungi or may promote fungal recovery. We conclude that incorporating fungi into restoration efforts may better facilitate the establishment of both belowground and aboveground components of ecosystems.

INTRODUCTION

Ecosystem degradation may fragment habitats and reduce biodiversity (Tilman et al. 1994, Hoekstra et al. 2005, Turner et al. 2007). Biodiversity loss and habitat fragmentation may impact ecosystem functioning by disrupting complex community interactions, including those between plants, animals, and fungi (Komonen et al. 2000). Fungi play a key role in regulating ecosystem function by maintaining biodiversity and plant productivity (van der Heijden et al. 1998), thus they are an essential component of ecosystem restoration. Nevertheless, to date, most restoration ecologists have not placed sufficient emphasis on addressing the impact of degradation on fungal communities nor on restoring plant-associated fungi in degraded ecosystems (but see Skujinš and Allen 1986, Allen et al. 2003, Maltz and Treseder 2015). Without explicit consideration of fungal communities, restoration ecologists may undermine successful restoration outcomes.

Fungi provide important benefits to ecosystems (Dighton 2016), which may be essential for improving restoration outcomes. For example, decomposer fungi can recycle plant litter and stabilize soils (Caesar-TonThat and Cochran 2000). Mycorrhizal fungi can provide nutrients to plants, which usually leads to increased photosynthetic rates and improved plant productivity (Treseder 2013). Diverse fungal communities may lead to better plant growth (Jonsson et al. 2001) and accelerated decomposition rates (Setälä and McLean 2004). Despite their pivotal role in the ecosystem, little is known about how fungi are affected by landscape degradation and the restoration methods used to combat it. We have only a limited understanding of how fungal responses to degradation and

restoration differ across ecosystem types and affect biotic communities and ecosystem processes.

Human-driven landscape degradation often separates biological communities and habitats into spatially distinct fragments (Preston 1962, Tilman et al. 1994, Haddad et al. 2015). This fragmentation not only reduces the size of a habitat but may also affect vegetation structure and native biodiversity within the fragment (Soulé et al. 1992, Young and Mitchell 1994, Rossetti et al. 2014, Wosniack et al. 2014). Fragmentation may reduce the number of different fungi (i.e., fungal taxa richness) within a fragment either directly by reducing suitable habitat, or indirectly via the effects of fragmentation on plants (Wardle et al. 2004, Ódor et al. 2006, Heilmann-Clausen et al. 2014). Since fragmentation spatially separates plant and fungal communities into habitats of different sizes, this may diminish the beneficial role of fungi within these ecosystems.

Invasions may be detrimental to native fungi as well (Schreiner and Koide 1993). For instance, invasive plants displace mycorrhizal host plants within fragmented habitats (Bever et al. 1996, Valyi et al. 2015). Additionally, some prominent invaders, such as the exotic forb *Brassica nigra,* may produce compounds that are toxic to both soil fungi and native plants (Lankau and Strauss 2008).

Although invasive plants may exacerbate degradation, common methods for their removal may extend beyond the plant community to affect soil biota (Irvine et al. 2013). Specific restoration methods may disturb soils and reduce invasive plant cover prior to restoring native plant cover (Greipsson 2011). These methods may thereby inhibit or promote fungi in invaded habitats (Binet et al. 2013, Druille et al. 2013). Restoration methods that affect fungi may have far-reaching consequences for ecosystems by

affecting fungal abundance (Maltz et al. in press) and nutrient cycling in invaded habitats (Suding 2011).

Even though ecosystem restoration methods rarely specify fungal restoration as a desired outcome, some restoration ecologists have directly manipulated fungal abundance by applying material containing fungi (i.e., mycorrhizal inoculum) (Sylvia 1989, Pineiro et al. 2013) to soil and plants as part of restoration. Additions of mycorrhizal inoculum may increase the abundance of mycorrhizal fungi in soil, which may also affect aboveground vegetation. In fact, direct mycorrhizal restoration may improve the mycorrhizal host plant's performance by increasing the percentage of root lengths colonized (PRLC) by mycorrhizal fungi. However, the efficacy of direct mycorrhizal restoration may not be equally effective across ecosystem types. Moreover, not all inocula are equivalent; different inocula types may yield unintended consequences for plant-fungal associations or plant growth in restored ecosystems. Inocula from neighboring reference ecosystems (i.e., ecosystems that exhibit characteristics intended of the restored ecosystem) may be better suited to local environments than exotic or commercial inocula. Therefore, refining strategies for direct mycorrhizal restoration in field trials may support best practices for restoring fungal communities and ecosystem function.

My dissertation examines the effects of habitat fragmentation and ecological restoration on fungal community composition and function. The goal of my dissertation work is to address the following questions:

(1) Does habitat fragmentation result in a loss of fungal function?

(2) Is fungal function in habitat fragments related to plant and fungal species richness?

(3) Do restoration methods reduce invasive plant cover, change mycorrhizal fungal abundance in soil, or promote the growth of native plants?

(4) Does direct mycorrhizal restoration result in sustained increases in PRLC and improved plant growth in field restoration?

(5) Do inocula sourced from reference ecosystems increase PRLC more than do commercial inocula?

In Chapter 1, I determine if habitat fragment size or the number of plant species in a fragment is related to the potential activity of litter-decay enzymes (as a measure of fungal function) (question 1). Also in Chapter 1, I describe a plant litter diversity manipulation to explore whether increased plant litter diversity accelerates decomposition and increases fungal taxa richness (question 2). I have written Chapter 1 with co-author Dr. Krista McGuire for submission to *Plant and Soil*. Chapter 2 discusses a manipulative field restoration project that investigates how common restoration methods affect invasive plant cover, mycorrhizal abundance in soil, and native plant communities in restored CSS ecosystems (question 3). The manuscript for my second Chapter is currently in press in *Ecological Restoration*. Chapter 3 is a meta-analysis of published restoration studies assessing whether direct mycorrhizal restoration increases PRLC and plant growth (question 4) in restored ecosystems. For Chapter 3, I synthesize findings from these studies across a variety of ecosystems to evaluate the efficacy of different inocula sources, inoculation methods, and durations of exposure to inoculum in the field

for the purpose of increasing PRLC and plant growth under field conditions (question 5).

This Chapter has already been published in *Restoration Ecology*.

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CHAPTER 1: Links between plant and fungal diversity in habitat fragments of coastal sage scrub

INTRODUCTION

Microbial processes regulate decomposition rates and the release of soil C pools into the atmosphere in the form of CO_2 (Bardgett et al. 2008). However, microbial processes are threatened by land-use changes (Flores-Renteria et al. 2016), which result in habitat fragmentation and biodiversity loss (Fischer and Lindenmeyer 2007, Malmivaara-Lamsa et al. 2007, Staddon et al. 2010). By physically separating biological communities into habitats of different sizes, fragmentation may exacerbate this biodiversity loss and alter ecosystem function (Ewers and Didham 2006, Butchart et al. 2010, Haddad et al. 2015). Although previous studies have shown that human-driven habitat fragmentation reduces biodiversity or ecosystem function, most of these studies have been conducted on large macroscopic organisms such as birds (Nol et al. 2005) or vascular plants (Ibanez et al. 2014) without sufficient emphasis on microbes (Burkey 1997, Riutta et al. 2012, Abrego et al. 2015). Fungi in particular are considered the engines of nutrient recycling and thus support numerous ecosystem functions (Hodge and Fitter 2010, Dighton et al. 2016). Given their functional role in the ecosystem (Christensen 1989, van der Heijden et al. 2008, Shay et al. 2015), it is important to understand more precisely how fragmentation affects fungi.

Determining how fungi respond to habitat fragmentation may be critical for managing fragmented ecosystems, yet our understanding of how habitat fragmentation may hinder fungal processes is relatively limited (Edman et al. 2004, Grilli et al. 2012). Previous studies have examined fragmentation effects on microbial communities using

experimental microcosms or experimentally fragmented mesocosms. These studies indicate that fragmentation can affect fungal growth rates (Bengtsson et al. 1993, Staddon et al. 2010) and that fragment size can influence rates of N cycling (Billings and Gaydess 2008). Although it is challenging for these microcosm or mesocosm experiments to fully replicate the complexity of fragmented ecosystems, these studies improve our ability to predict how habitat fragmentation may affect fungal function.

Fragmentation often limits the number of plant species (i.e., plant species richness) in small fragments (Soule et al. 1992, Honnay and Jacquemyn 2007, Ibanez et al. 2014); these reductions in plant diversity may in turn affect plant litter and fungal function. Fungi and the extracellular enzymes they produce serve important ecological functions; these enzymes are considered to be rate-controlling agents of decomposition (Sinsabaugh et al. 2002). Decomposition of plant litter is driven by a successional loop in which fungal communities and extracellular enzymes are linked to plant litter substrates (Sinsabaugh 2005, Allison et al. 2007). In this loop, the composition of plant litter substrates may select for fungal taxa that produce extracellular enzymes which modify a particular substrate. The downstream products and byproducts of degradation may likewise select for different taxa capable of modifying other substrates within decaying litter. Because plant species differ in their litter chemistries and decompose at different rates, diverse plant litter mixtures are likely to contain a variety of substrates at different stages of decay at any given time. This increased variety of substrates (Abrego and Salcedo 2014) and the complex interactions among both enzymes and substrates (Sinsabaugh 1991) may not only enhance potential extracellular enzyme activity (EEA), but may also increase microhabitat heterogeneity and diversify fungal growth forms

(Hansen and Coleman 1998). In fragmented habitats, with fewer numbers of plant species, there may be only a narrow range of litter substrates for fungi, which may in turn affect fungal activity or inhibit fungal processes. Thus, human-driven habitat fragmentation may not only alter litter composition by reducing plant diversity (Soulé et al. 1992) but may also affect fungal enzyme activity by changing the functional capacity of fungal communities (Hui et al. 2007).

However, the relative influence of plant species richness on fungal function may be difficult to isolate from the effects of habitat fragment size on fungal function. In fact, the consequences of reduced plant diversity in a small fragment may be as important as the size of that fragment in determining fungal function. Elucidating the mechanisms driving fungal function may be crucial for managing habitat remnants within fragmented ecosystems.

Because California's coastal sage scrub (CSS) ecosystems are a prime example of fragmentation (Vandergast et al. 2007), evaluating the impacts of habitat fragmentation and reduced plant diversity on fungi in CSS ecosystems would be especially valuable. These CSS ecosystems are the second most extensive native ecosystem type in California, yet CSS habitats are becoming increasingly rare owing to urban and agricultural development and anthropogenic N deposition (Fenn et al. 2003, Leyva et al. 2006, Syphard et al. 2007). In fact, only about 10% of CSS ecosystems are still intact (McCaull 1994), making these plant communities arguably the most endangered habitat in the United States (Rubinoff 2001). Fragmentation affects CSS-associated biotic communities (Vandergast et al. 2007), but the linkages between CSS plant diversity and

fungal function remain poorly understood. Therefore, understanding fungal dynamics in CSS habitats is especially important for managing these fragmented ecosystems.

In order to examine the effects of habitat fragmentation on plant species richness and fungal function, and to evaluate the extent to which fungi are influenced by plant species richness in fragmented ecosystems, we addressed the following questions: (1) Does habitat fragmentation reduce plant diversity and fungal function? (2) Does plant species richness control fungal taxa richness and fungal function?

To answer these questions, we investigated the effects of habitat fragment size on plant richness and fungal function in fragmented CSS ecosystems. In order to evaluate fungal function, we quantified the potential activity of three extracellular enzymes important in either chitin or cellulose degradation within plant litter from habitat fragments of varying sizes. Next, we manipulated plant litter richness in order to isolate the effect of reduced plant species richness on fungal taxa richness and decomposition. We hypothesize that (1) plant species richness and fungal function decline with habitat fragment size and that (2) plant litter diversity controls fungal taxa richness and fungal function in fragmented CSS habitats.

MATERIALS AND METHODS

Site descriptions

We investigated the effects of habitat fragmentation and plant richness on fungal function in a native, fragmented, CSS habitat at Newport Back Bay in southern California (33° 37’ 35” N, 117° 53’ 30” W). Soils at our study sites are classified as Typic Palexeralfs and Typic Xerorthents belonging to the Myford and Cieneba series (Schimel

and Chadwick 2013). Soils are moderately to excessively well-drained sandy loams with neutral to slightly acidic pH (~ pH 6.0 - pH 6.8).

Dominant shrub species are *Peritoma arborea* (California bladderpod), *Artemisia californica* (California sagebrush), *Encelia californica* (California brittlebrush), *Rhus integrifolia* (lemonade berry), *Eriogonum fasciculatum* (California buckwheat), *Isocoma menziesii* (coast goldenbush), and *Salvia apiana* (white sage). Non-native plants are present in areas adjacent to our fragments, including *Brassica nigra* (black mustard), *Salsola kali* (Russian thistle), *Cynara cardunculus* (artichoke thistle), and *Schinus terebinthifolius* (Brazilian peppertree). Surrounding these fragments are open grounds and walking trails. This site experiences a Mediterranean climate with warm dry summers and cool wet winters. The mean annual temperature is 16.6 °C. Mean annual precipitation is 295 mm with the majority of precipitation occurring between November and March (http://www.nws.noaa.gov/).

Fragment size

To investigate the effects of habitat fragmentation on fungal function, we established plots within a series of six CSS fragments. The fragments in our study were composed of one relatively large fragment (5415 m^2) and five smaller fragments (23 m^2, 26 m^2 , 186 m^2 , 684 m^2 , and 1234 m^2). Plant species richness in each fragment increased with fragment size (Fig. 1). We created five 5 m transects in each fragment and randomly collected ten surface litter samples from each transect. Samples were immediately frozen on dry ice for transport back to the laboratory at University of California, Irvine. Samples were thawed and composited in the laboratory. We assessed EEA in each composited litter sample by conducting assays on 0.1 g litter samples from each fragment.

From each litter sample, we assessed EEA for the chitinase *N*-acetyl-glucosaminidase (NAG) and two cellulase enzymes, β-glucosidase (BG) and cellobiohydrolase (CBH), involved in plant litter degradation using the protocol of Alster et al. (2013) (Table 1.1). We added 0.1 g of litter to 60 ml of 25 mM maleate buffer solution (pH 6.0). To create homogenate solutions, we homogenized our litter samples using a Biospec Tissue Tearer (14 mm generator) for four 30 second pulses with 30 second intervals between each pulse. Then we pipetted 200 ml of stirred homogenate per well into eight replicate wells within a 96-well microplate. We added 50 µl of 1000 µM substrate solutions to each homogenate well; each substrate solution was prepared from 4-methylumbelliferone (MUB) fluorescent dye-conjugated substrates specific to each enzyme (Table 1.1). We corrected for background fluorescence with homogenate control wells (without substrate solutions added) and substrate control wells (without added homogenate samples). Black micro-plates were covered for one hour; after incubation, we added 10 µl of 1.0 NaOH to stop reactions.

We conducted fluorimetric assays (as detailed in German et al. (2011) for each of three hydrolytic enzymes: BG, CBH, and NAG. We measured fluorescence at 365 nm excitation and 450 nm emission. From each sample, we recorded fluorescence values for MUB substrate (substrate control), homogenate (homogenate control), MUB standards in the presence of maleate buffer (standard), and MUB in the presence of homogenate. We calculated potential EEA per Alster et al. (2013) as:

$$\text{Activity (nmol } g^{-1}h^{-1}) = \frac{\text{Net fluorescence} \times \text{Buffer volume (ml)}}{\text{Emission coefficient} \times \text{Homogenate volume (ml)} \times \text{Time (hours)} \times \text{Litter mass (g)}}$$

Decomposition experiment

Our plant litter manipulation experiment allowed us to isolate the effects of plant diversity on fungal taxa richness and function from the effects of fragment size on these response variables. We experimentally manipulated plant litter diversity on the soil surface in our largest CSS fragment using a mixture of plant species that were representative of those found in the fragments (Table 1.2). For this experiment, we constructed 20 cm x 20 cm mesh bags from 1 mm nylon mesh (i.e., litter bags; Millipore, Bradford MA, USA) and we added 2 g of air-dried leaf litter collected from our fragments into each bag. We randomly assembled the leaf litter into mixtures composed of either one single plant species, three mixed species, five mixed species, or seven mixed species. Each single-species litter bag contained only one of each of the seven plant species. The mixed-species litter bags contained randomized mixtures of plant litter; the numbers of plant species in each of our mixed-species litter bags were representative of a plant diversity level found in one of our habitat fragments. Next, we sterilized the leaf litter via gamma irradiation (UC Irvine Medical Center, Irvine CA) at 2.5–3.0 Mrad for 48 hours. We cleared all standing litter from a 2 m^2 randomly-located plot within the largest habitat and deployed these litter bags in a randomized block design with seven replicates per plant litter diversity level, resulting in a total of 28 litter bags within the plot. These nylon litter bags allowed nutrients, microbes, fungi, and micro-fauna to move freely into and out of the litter bags.

After one year of field incubation, we harvested the litter bags. Then, we weighed each litter bag to determine the percent leaf litter mass remaining in each bag. As a measure of decomposition, we calculated percent litter mass remaining (%) as the mass

of oven-dried litter (g) in each bag after one year of field incubation divided by the initial mass of dried litter (g) in that litter bag.

Litter mass was then subsampled to assay for fungal taxa richness as per McGuire et al. (2013). We weighed 0.25 g aliquots of each litter sample in triplicate. We extracted total DNA from each of the three 0.25 g litter aliquots using a Powersoil DNA extraction kit (MoBio Carlsbad, CA) and pooled these DNA extracts into a single representative DNA extract. DNA concentrations were standardized to 10 ng/µl before PCR amplification. We targeted a portion of the 18S rRNA gene using universal fungal primers modified for 454 pyrosequencing, with unique molecular barcodes assigned to each reverse primer (Borneman and Hartin 2000, Lauber et al. 2009). Fungal-specific DNA was amplified (SSU 817f-1196r) using 0.25 µl of each primer (30 µM), 1.0 µL of BSA (10 mg mL-1), 3.0 µl of DNA template, and 22.5 µl Platinim PCR SuperMix (Invitrogen, Carlsbad, CA). Reactions ran for 30 cycles of 94° C for 45 seconds, 52° C for 30 seconds, and 72° C for 90 seconds with a hot start at 94° C for 10 minutes and final extension step at 72° C for 10 minutes. We pooled three PCR reactions for analysis, purification, and quantification; purified PCR products were then combined into an equimolar solution for downstream DNA pyrosequencing.

Our PCR products were sequenced by the Environmental Genomics Core at the University of South Carolina (Columbia, SC) on a Roche 454 Gene Sequencer (Roche 454 Life Sciences, Branford, CT) using titanium chemistry. We used a high-throughput pyrosequencing protocol and bioinformatics pipeline described in detail in McGuire et al. (2013) for analyzing small-subunit rDNA of fungal communities. Sequenced amplicons were aligned and grouped into operational taxonomic units (OTUs) (i.e., fungal taxa) and

taxa were defined as DNA sequences sharing ≥97% sequence identity; taxon level for this targeted portion of the small-subunit 18S rRNA gene has resolution at the fungal family level. Taxonomic information for each fungal taxon was determined using the BLAST algorithm (Altschul et al. 1997) against identified sequences in Genbank and SILVA databases. For quality control purposes, we discarded any sequences < 400 bp and sequences with a phred quality score < 25. Samples were normalized to 562 sequences per sample, and any samples with fewer than 562 sequences or with unreadable barcodes were removed from our downstream analysis. Additionally, non-fungal sequences were manually removed following taxonomic assignment.

Statistical analysis

Fragment size

To test our prediction that plant species richness declines with habitat fragment size, we performed a linear regression with fragment size as the independent variable and the number of plant species in a fragment as the dependent variable. If the number of plant species significantly decreased along with declining fragment size, this would support our first Hypothesis.

We evaluated whether fungal function declines with either fragment size or with the number of plant species in each fragment. In each of these cases, the independent variable was either fragment size or the number of plant species in a fragment, and the dependent variable was litter EEA. Any significant decline in fungal function could be driven either directly by fragment size, or indirectly by plant species richness. Analyses associated with our plant litter manipulations enabled us to isolate the influence of plant diversity on fungal diversity and function.

Decomposition experiment

We conducted linear regressions in order to address our second Hypothesis that plant litter diversity controls fungal taxa richness and fungal function in fragmented CSS habitats. In each case, the independent variable was plant litter richness, and the dependent variables were either fungal taxa richness or percent litter mass remaining. Either a significant decline in fungal taxa richness with decreasing litter richness or a significant increase in the percent remaining of litter mass with decreasing litter richness would support our second Hypothesis. Additionally, we examined whether fungal diversity is related to decomposition. In this additional case, the independent variable was fungal taxa richness and the dependent variable was percent litter mass remaining. A significant increase in percent litter mass remaining with decreasing fungal taxa richness would suggest that fungal taxa richness may be related to fungal functions like decomposition. If reduced plant species richness contributed to diminished fungal diversity and function, then this may indicate that fragmentation effects on plants extend beyond the plant community.

RESULTS

Fragment size

The species richness of plants in each fragment was correlated with fragment size ($R^2 = 0.976$, $p < 0.001$, Fig. 1.1). Furthermore, we observed that fungal function varied by fragment size and by plant species richness in the fragments. Specifically, litter EEA was positively correlated with fragment size for three extracellular enzymes: BG ($R^2 = 0.712$, $p = 0.035$), CBH ($R^2 = 0.766$, $p = 0.022$), and NAG ($R^2 = 0.934$, $p = 0.002$) (Fig.

1.2). Altogether, these results supported Hypothesis 1 in that plant species richness and fungal function declined with fragment size. Additionally, litter EEA was positively correlated with plant species richness in the fragments for these same extracellular enzymes: BG ($R^2 = 0.795$, $p = 0.017$), CBH ($R^2 = 0.853$, $p = 0.008$), and NAG ($R^2 = 0.981$, $p < 0.001$) (Fig. 1.3).

Decomposition experiment

After one year, litter bags containing more species of plant litter had less litter mass remaining than litter bags with fewer plant species ($R^2 = 0.721$, $p = 0.001$, Fig. 1.4). Litter bags with more plant species yielded significantly greater fungal taxa richness than litter bags with fewer plant species ($R^2 = 0.639$, $p = 0.003$, Fig. 1.5). These findings support our second Hypothesis in that plant litter diversity controlled both fungal taxa richness and fungal function in our litter bags. A majority of fungal taxa identified from our litter bags were dikaryotic fungi, with 80% of fungal OTUs from Ascomycota and 13% of OTUs from Basidiomycota; the remaining 7% of OTUs were zoosporic taxa from either Chytridiomycota or Blastocladiomycotina. Our findings suggest that increased decomposition may have occurred in litter bags with greater numbers of fungal taxa than in those with fewer fungal taxa, as demonstrated by less mass remaining in these litter bags that contained more fungal taxa ($R^2 = 0.516$, $p = 0.012$, Fig. 1.6).

DISCUSSION

We examined the effects of habitat fragmentation on fungal function in a fragmented CSS ecosystem and found reduced fungal function in smaller habitat fragments. We found that fungal function is related to both fragment size and plant

diversity. Moreover, by manipulating plant litter diversity, we were able to isolate the effects that plant species richness had on fungal taxa richness and decomposition. In our plant litter manipulation study, we found that plant diversity controlled both fungal taxa richness and fungal function in plant litter. Our findings suggest that the size of a fragment may indirectly influence fungal function via changes in plant diversity.

Fragment size

Habitat fragment size is negatively related to plant species richness (Fig. 1.1). As intact CSS ecosystems in southern California shrink, certain CSS plants may compete for the limited habitat found within small fragments (Ives 1995, Wosniack et al. 2014). Therefore, habitat fragmentation may result in local extinction, or extirpation, of plant species via competitive exclusion (Soulé et al. 1992, Raghubanshi and Tripathi 2009). This process of competitive exclusion suggests that reduced niche space in smaller fragments may constrain the plants in those fragments (Amarasekare et al. 2003), which may in turn affect fungal function.

Overall, we found that fungal function significantly declined with fragmentation of the CSS ecosystem. Specifically, we observed reduced chitinase (NAG) activity in litter from smaller fragments (Fig. 1.2). Chitin is a polysaccharide present in fungal cell walls that is produced during fungal growth (Wessels 1986, Haran et al. 1995, Baldrian 2008); NAG may mineralize N from this chitin (Olander and Vitousek 2000). Previous studies have found NAG activity to be correlated with fungal biomass in plant litter (Rodriguez-Kabana et al. 1983, Talbot and Treseder 2012). In our study, the observed decline in NAG activity within litter from small fragments could indicate that either

fungal growth, fungal turnover, or fungal function was diminished in CSS fragments that contained fewer numbers of plant species (Fig. 1.3).

We also observed that litter cellulase activity decreased with smaller CSS fragments (Fig. 1.2) and with decreasing numbers of plant species (Fig. 1.3). Fragments with fewer plant species may produce litter containing a limited variety of cellulose or other structural polysaccharides. Low-diversity litter with fewer types of cellulose may have diminished substrate availability for cellulolytic enzymes (Wilson 2011), and this may partially explain why we observed less cellulase activity in litter from smaller fragments. Limited substrate availability may likewise inhibit the cellulytic potential of fungal communities by hindering cellulase gene expression (Berlemont et al. 2014). Since these enzymes (BG, CBH) are important for hydrolyzing compounds within cellulosic plant litter (Ljungdahl and Eriksson 1985, Sinsabaugh et al. 2008), this decrease in litter EEA may thus have inhibited cellulose degradation in litter from small habitat fragments with less diverse plant communities.

Both plant litter resources and fungal function may be affected by habitat fragmentation's constraints on plants because plant species differ in their chemistry and structure. Decomposing material from less diverse plant communities may contain a narrow range of resources (Gartner and Cardon 2004), which could limit substrate availability and litter quality. The nutrient dynamics within less diverse or low-quality litter could potentially inhibit fungal function (McTiernan 1997). Considering the high N cost and energy intensive process of enzyme synthesis, nutrient limitation may either constrain or promote EEA; these resultant outcomes may depend on the particular enzymes or an availability of both complex substrates and assimilable resources (Harder

and Dijkhuizen 1983, Allison 2005, Allison and Vitousek 2005). Yet, our findings suggest that litter resources from small CSS fragments may constrain the successional loop among microbial communities, extracellular enzymes, and substrates (Sinsabaugh et al. 2002). Constraints on plants in small fragments may therefore lower EEA and disrupt interactions among fungi and plant litter substrates. In other words, small fragments characterized by less diverse resources may be less apt to support functionally diverse microbial communities.

Decomposition experiment

The effects of fragmentation on plant diversity may extend to soil biota (Ashford et al. 2013). Indeed, we found that direct manipulations of plant litter diversity affected fungal taxa richness (Fig. 1.5). This suggests that reductions in plant diversity following fragmentation, rather than fragmentation itself, may be the proximate cause of changes in the fungal community.

Reductions in plant diversity may change fungal communities either by reducing ecological niche space or by changing plant litter quality (Wardle et al. 2004). Diverse plant communities may provide more niche space for fungal taxa that exploit specialized ecological niches (Nordén et al. 2013). Highly diverse communities may not only contain more functionally redundant taxa (Walker 1992, Allison and Martiny 2008), but may also host a range of species that respond differently to disturbance and thus help stabilize ecosystems (Hooper et al. 2005, Shade et al. 2012). Therefore, plant diversity may even serve as a form of biological insurance (Naeem and Li 1997) by preventing losses of specialist or rare fungal taxa from fragmented ecosystems. Altogether, communities with

greater plant diversity should produce more diverse litter resources and provide more ecological niche space, which may support greater numbers of fungal taxa.

Following our decomposition experiment, less litter mass remained within litter bags containing diverse plant litter (Fig. 1.4) and more fungal taxa (Fig. 1.6). This suggests that these diverse fungal assemblages may have consumed more resources from mixed-litter bags than from single-species litter bags. This may have occurred as a consequence of increased fungal diversity in these litter bags, as highly diverse communities are more likely to contain species that use resources efficiently (Cardinale et al. 2006). Alternately, sampling effects could result in litter mixtures containing more productive plant species (Tilman et al. 1997) or plant species that rapidly decompose (Hector et al. 2000); this could contribute to increased decomposition in more diverse litter mixtures as compared to less diverse or single species litter bags. However, more diverse litter mixtures also contained greater numbers of fungal taxa, which suggests that there may have been synergistic effects among plant litter richness and fungal taxa richness on decomposition. As fungal taxa specialize on particular organic substrates, increased fungal diversity may consequently accelerate decomposition rates (McGuire et al. 2010).

Our study provides evidence that both habitat fragment size and the number of plant species in a fragment affect fungal function, yet it has some limitations. For instance, our study was conducted within only one ecosystem, and thus our interpretations are limited to CSS ecosystems. Additionally, while we opted to focus our study on fungi, other microbes like bacteria contribute to soil processes in fragmented habitats (Burkey 1997, Riutta et al. 2012). Beyond fungi, investigating other microbial

responses to fragmentation was outside the scope of this study. Nevertheless, because fungi play a key role in biogeochemical cycles (Dighton 2016, Gadd 2006), determining their functional response is especially important for predicting how CSS ecosystems respond to fragmentation. It is worth noting that other factors related to plant richness may independently influence fungal function; however, results from our plant litter manipulation suggest that fungal taxa richness may play a role in litter decomposition in remnant CSS habitats.

Conclusion

Our results suggest that reduced plant diversity may constrain both fungal taxa richness and fungal function in fragmented CSS ecosystems. We found that plant diversity in CSS ecosystems was particularly susceptible to fragmentation, which may ultimately limit fungal metabolic activities. Larger fragments containing multiple litter types may provide sufficient microhabitat heterogeneity for supporting greater numbers of functionally diverse fungal taxa. These diverse fungal communities may efficiently exploit litter resources, which may increase decomposition. Therefore, as fragmentation directly reduces plant diversity it may also indirectly inhibit fungal function. Altogether, our findings provide evidence that even fungi may be affected by human-driven habitat fragmentation via direct effects of fragmentation on plants. These data have crucial implications for management of CSS ecosystems. For instance, restoration methods that aim to restore diverse vegetation communities within larger fragments may be especially effective at improving the functional capacity of degraded sites. This study underscores

the importance of both reducing habitat fragmentation and maintaining diversity when restoring ecosystems.

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TABLES

Table 1.1 The extracellular enzymes assayed from litter from a fragmented CSS ecosystem; abbreviations, functions, and corresponding substrates for these enzymes.

Enzyme	Abbreviation	Function	Substrate
β-glucosidase	BG	Cellulose degradation	4-MUB-β-D-glucopyraniside
Cellobiohydrolase	CBH	Cellulose degradation	4-MUB-β-D-cellobioside
N-acetyl-β-D-glucosaminidase	NAG	Chitin degradation	4-MUB-N-acetyl-β-D-glucosaminide

Table 1.2 Native plant species in our litter bags

Enzyme	Function
Artemisia californica	California sagebrush
Eriogonum fasciculatum	California buckwheat
Encelia californica	bush sunflower
Peritoma arborea	California bladderpod
Rhus integrifolia	lemonade berry
Isocoma menziesii	Menzies' goldenbush
Salvia apiana	white sage

FIGURES

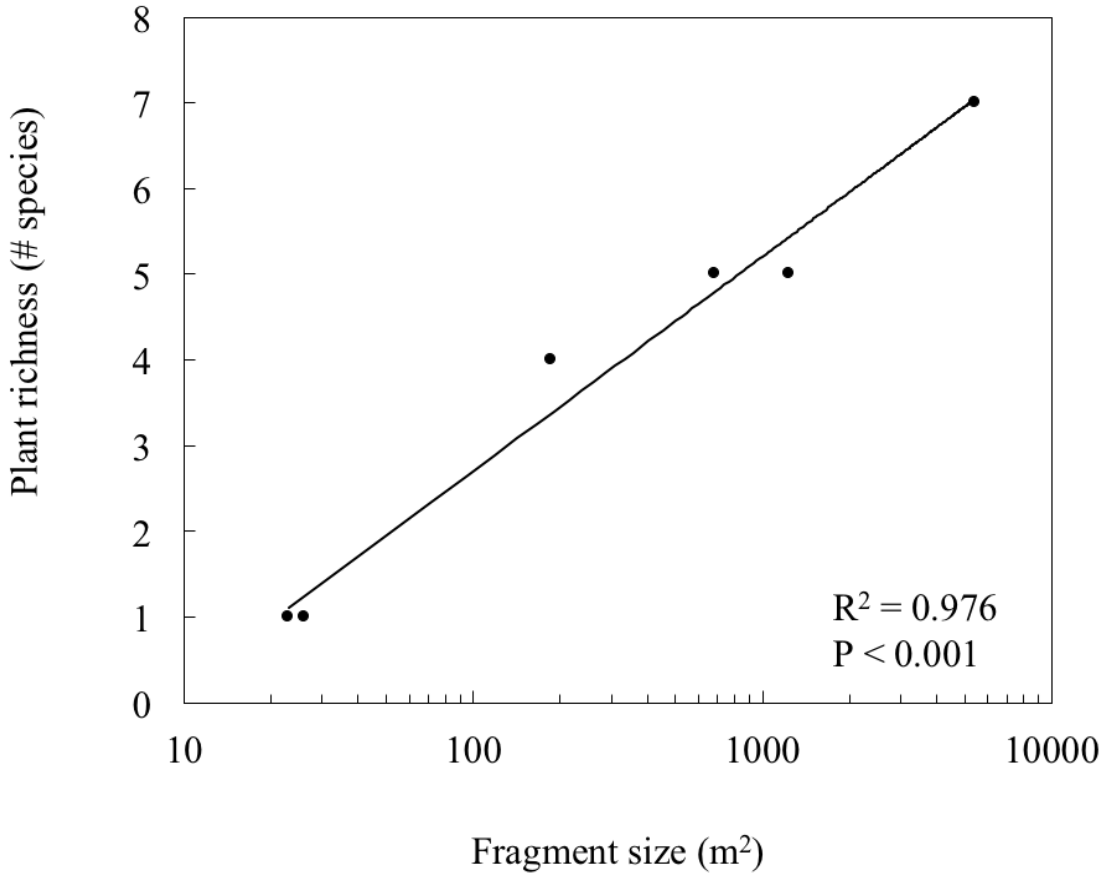

Figure 1.1. Plant species richness (number of plant species in a fragment) significantly increased with increasing log fragment sizes (m^2) ($p < 0.001$). The best-fit lines are shown with corresponding R^2 values.

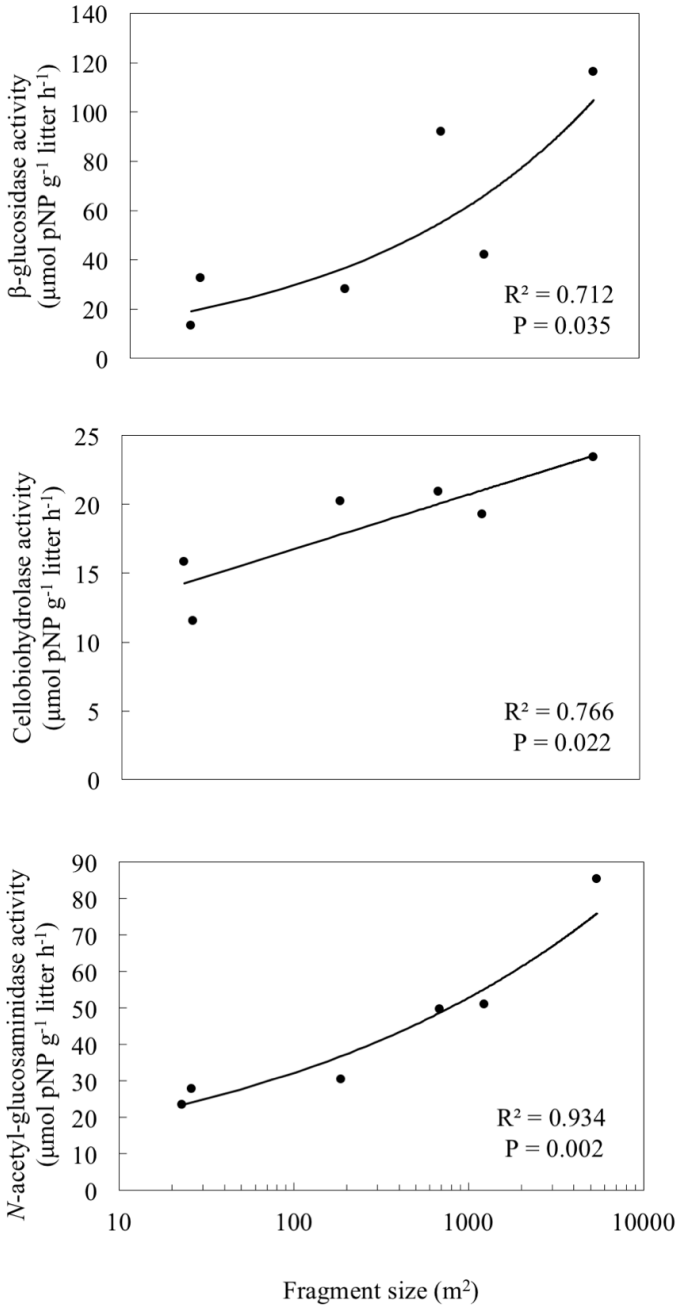

Fig. 1.2. Extracellular enzyme activity (μmol pNP g^{-1} litter h^{-1}) in litter was significantly correlated with log fragment size (m^2) for three extracellular enzymes involved in cellulose (β-glucosidase, p = 0.035; cellobiohydrolase, p = 0.022) and chitin (*N*-acetyl-glucosaminidase, p = 0.002) degradation. The best-fit lines are shown with corresponding R^2 values.

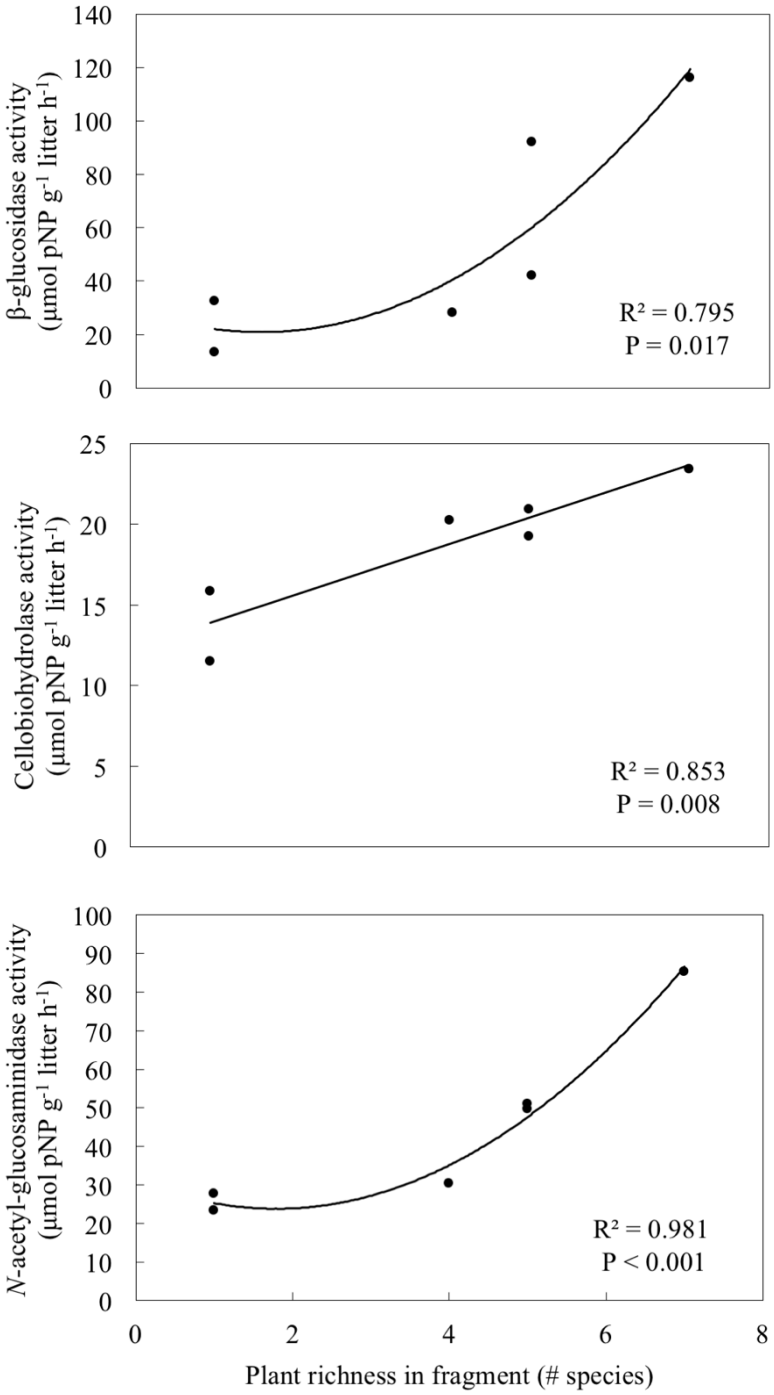

Fig. 1.3. Extracellular enzyme activity (μmol pNP g^{-1} litter h^{-1}) in litter was significantly correlated with plant richness in the fragments for three extracellular enzymes involved in chitin and cellulose degradation. (β-glucosidase, p = 0.017; cellobiohydrolase, p = 0.008; *N*-acetyl-glucosaminidase, p < 0.001). The best-fit lines are shown with corresponding R^2 values.

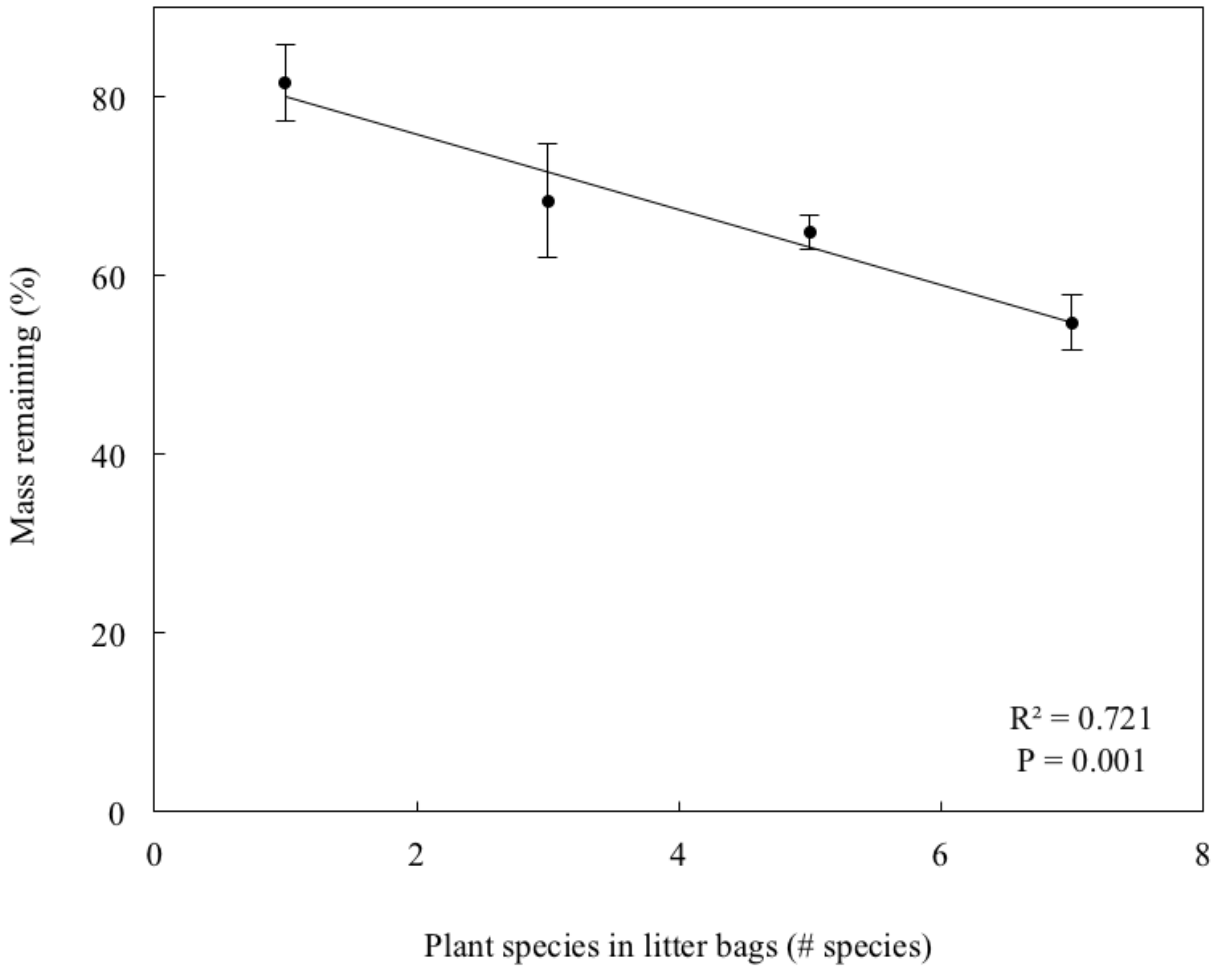

Fig. 1.4. Percent mass remaining of litter bags containing different levels of plant litter richness after one year. Following field incubation, litter bags containing more species of plant litter had less plant litter mass remaining than litter bags with fewer plant species (P = 0.001). The mean percent mass remaining ±1 SE are shown for each litter diversity manipulation.

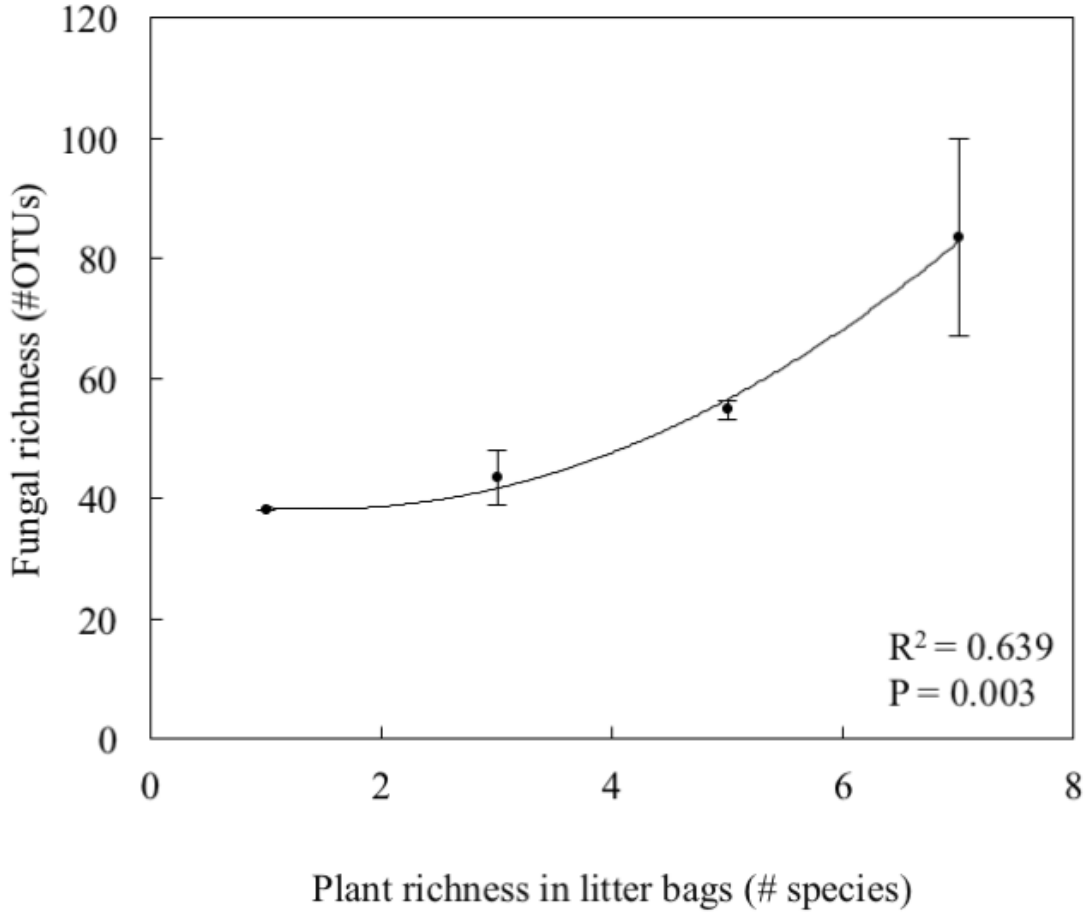

Fig. 1.5. Fungal richness (#OTUs) increased with greater number of plant species (# species) represented within litter bags ($p = 0.003$). Symbols represent means (±1 SE) of fungal taxa richness within our plant litter diversity treatments.

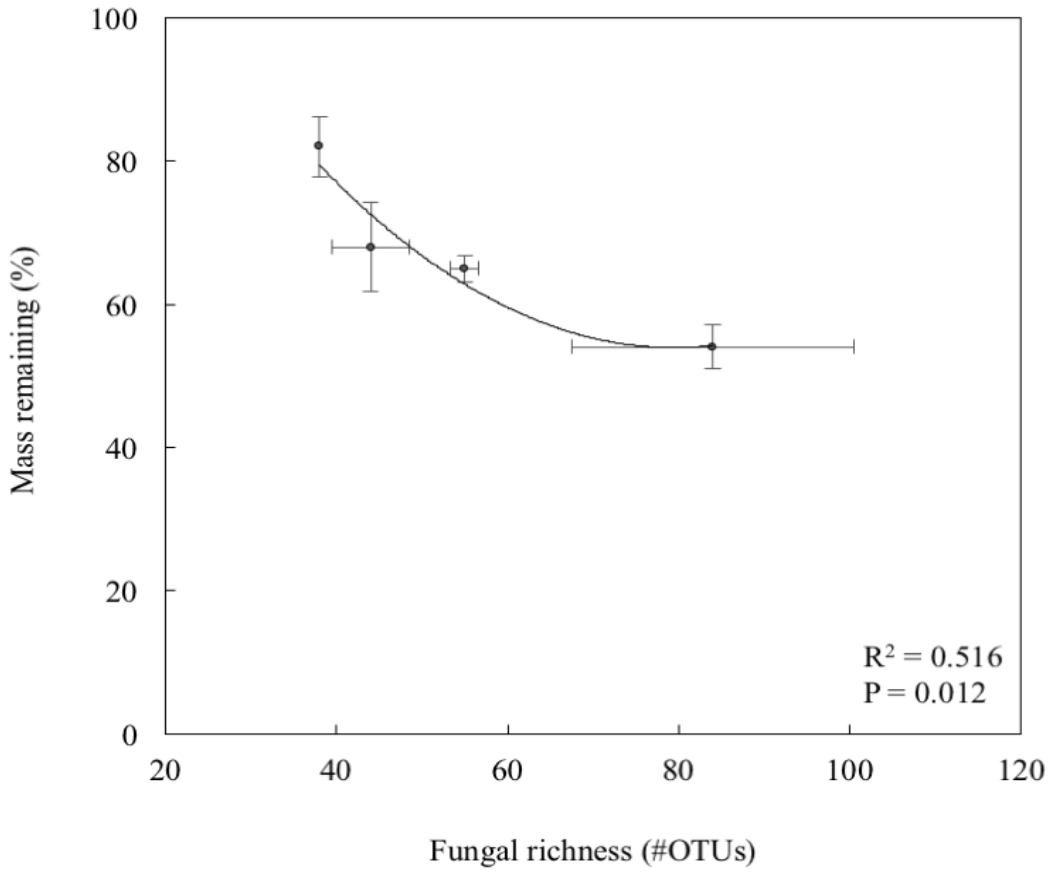

Fig. 1.6. Mass remaining (%) in litter bags was related to the number of fungal taxa (p = 0.012) in these litter bags. Symbols represent means (±1 SE) of mean fungal taxa richness and mean percent mass remaining within our plant litter diversity treatments.

CHAPTER 2: Invasive plant management techniques alter arbuscular mycorrhizal fungi

INTRODUCTION

Arbuscular mycorrhizal (AM) fungi perform essential ecosystem services by improving plant growth and biodiversity (van der Heijden et al. 1998, Auge 2001, Hoeksema et al. 2010) and by deterring erosion (Rillig et al. 2015). Thus, they can be "hidden players" in ecological restoration (Error! Hyperlink reference not valid., Allen 1989, Allen et al. 2003). Unfortunately, ecosystem degradation can reduce the abundance of AM fungi (Allen and Allen 1980, Allen and MacMahon 1985, McGonigle & Miller 1996), which may limit restoration success (Maltz and Treseder 2015). Restoration often strives to rehabilitate native vegetation (Greipsson 2010), but certain practices may inadvertently harm (or help) AM fungi (Ingram et al. 2008, Druille et al. 2013b). Indeed, we know little about how AM fungi are affected by restoration methods that target plants.

Invasive plants are a common source of ecosystem degradation (Vitousek et al. 1997, Kimball et al. 2014). Among other things, invasive plants can alter soils and influence belowground communities (e.g., Vitousek & Walker 1989). For instance, one prominent invader of Californian coastal sage scrub, *Brassica nigra* (black mustard), releases glucosinolates from roots into the soil (Raybould & Moyes 2001, Lankau & Strauss 2007). Glucosinolates are allelopathic compounds that inhibit the growth of AM fungi (Schreiner & Koide 1993, Lankau & Strauss 2007). Moreover, black mustard is non-mycorrhizal (Pendleton & Smith 1983, Crowell & Boerner 1988, Kliebenstein 2004, Lankau 2012). Thus, AM fungi may decline in black-mustard invaded grasslands and coastal sage scrub for two reasons: the presence of allelochemicals and the displacement

of native AM host plants. Thus, removal of black mustard could potentially improve AM growth by off-setting these negative effects.

On the other hand, the use of herbicide and mowing to remove black mustard might directly harm AM fungi. The herbicide glyphosate can directly—and strongly—decrease AM root colonization, propagules, and spore viability (Druille et al. 2013a, Druille et al. 2013b, Zaller et al. 2014, Druille et al. 2015), although not in every case (Malty et al. 2006, Pasaribu et al. 2013). Furthermore, glyphosate is a broad-spectrum herbicide, which may reduce native AM host plant abundance if applied after native seeds have germinated. Additionally, mowing can physically disrupt the soil surface (Greipsson 2010), which may damage AM mycelia. Moreover, mowing can also potentially remove neighboring native plants that serve as AM hosts (Bever et al. 1996, Vályi et al. 2015). Yet, we do not know if potential damage from herbicide or mowing is large enough to negate potential gains owing to black mustard removal, or how the effects of these two techniques compare to one another.

Here, we examine effects of herbicide and mowing on AM fungi to assess best practices of invasive plant removal for facilitating this important fungal group. We addressed these issues in a restoration experiment in Southern Californian coastal sage scrub that was heavily invaded by black mustard. Southern California is a biodiversity hot spot since its native ecosystems harbor an exceptionally large number of endemic plant species (Vandergast et al. 2008). Thus, restoration work is critical for this region. In this experiment, mowing and glyphosate were applied separately to remove black mustard. We tested three hypotheses: herbicide and mowing would each (1) reduce black

mustard cover; (2) change AM abundance in soil; and (3) promote the growth of native AM host plants accordingly.

MATERIALS AND METHODS

Field sites

We tested our hypotheses in an experimental restoration project established in two coastal sage scrub sites in Orange County. One was located in Crystal Cove State Park (33.5642° N, 117.8147° W), and the other in the University of California Irvine (UCI) Ecological Preserve (33.6412° N, 117.8447° W). At the time of sampling, the Nature Reserve of Orange County (now known as the Natural Communities Coalition) and the University of California Cooperative Extension had maintained the experiment for four years. The Crystal Cove site is very near the Pacific Ocean, about 2.5 km inland. The UCI site was farther inland, about 5.5 km from the coast. Coastal fog is more common in the Crystal Cove site, but otherwise the two sites have similar climates. Mean annual temperature is 17.5 °C; mean annual precipitation was 366 mm y^{-1} (NOAA, www.weather.gov). The climate is Mediterranean, with most precipitation occurring as rain from November to April. Soils at both sites are clay-loam, but the Crystal Cove sites have more loam and the UCI sites have more clay. Dominant shrubs at each site include *Baccharis pilularis, Eriogonum fasciculatum,* and *Encelia californica.* Although the study sites were invaded by black mustard, these invasive plants were not previously managed at our treatment plots.

Experimental Design

The Crystal Cove site contains four blocks, located in four areas across Crystal Cove State Park. The UCI site contains two blocks, located within the UCI Ecological Preserve. Each block consists of three treatment plots: one plot was mowed, one received herbicide, and one remained unmanipulated as a control. Altogether, there were six replicate plots per treatment across both sites, for a total of 18 plots. Mowing (with thatch removal) and herbicide applications (broadcasts of 1.68 kg/ha of broad-spectrum glyphosate in a spray volume of 375 l/ha) were applied in February or March of each year. Applications occurred after black mustard seeds had sprouted, but before most native forb and shrub seeds had germinated (Bell et al. 2016). Treatments were applied each spring from 2010 through 2013.

Plant Cover

Between April 11th and April 18th, 2013, we measured black mustard plant cover and native plant cover. Specifically, in each plot, we used a 1 m^2 quadrat to determine presence or absence of black mustard or native plants at 84 random locations (Table 1). In addition, we summed the percent cover of all native plant species.

Soil Collection

On July 8–12, 2013, we collected soils from each treatment plot. Three 9 cm diameter × 12 cm deep soil cores were collected and composited from each treatment plot, totaling 18 soil samples. Surface litter and debris were removed before coring. Samples were immediately placed on dry ice for transportation to the laboratory at UCI. Soils were used to measure AM abundance.

AM Abundance

We extracted fungal hyphae from each soil sample, and then quantified AM hyphal length as an index of AM abundance. We used a modified procedure from Sylvia (1992) and Brundrett et al. (1994). Specifically, we extracted 4 g of soil (fresh weight) with a 1.5 M solution of sodium hexametaphosphate. We passed this soil solution through a 0.2-µm nylon filter. Hyphae were collected on the filter. We stained the hyphae by immersing the filter in 0.01% (w/v) acid fuchsin dye. Filters were allowed to air dry for 15 minutes at room temperature, on a glass microscope slide, after which cover slips were affixed using polyvinyl alcohol/lactic acid glycerol medium (Koske and Tessier 1983). Slides were dried at 60° C for a minimum of 24 hrs. Then we examined each slide at 40x magnification using a phase contrast compound microscope (Nikon, Melville, NY). Arbuscular mycorrhizal hyphal lengths were quantified by examining each filter at 100 random locations for the presence or absence of AM hyphae. Arbuscular mycorrhizal hyphae were distinguished from other fungal hyphae based on morphology. Generally, AM hyphae feature angular branching, lack of septa, and irregular walls (Bonfante-Fasolo 1986).

Statistics

We conducted a fully-factorial analysis of variance (ANOVA) to test for effects of restoration treatment (independent variable) and site (independent variable) on AM hyphal lengths (dependent variable). Tukey's post hoc tests for pairwise differences were applied as appropriate. We arcsine-transformed hyphal lengths to improve normality and homogeneity of variances (Sokal and Rohlf 1995). For black mustard cover, we performed a one-way chi-square analysis with treatment as the independent variable,

another one-way chi-square analysis with site as the independent variable, and a two-way chi-square analysis with treatment and site as two independent variables. Where indicated, we conducted post hoc pairwise comparisons between treatments. In each case, the dependent variable was percent cover of black mustard. For native plant cover, we performed the same series of chi-square analyses, but with percent cover of native plants as the dependent variable. We used Systat 13 for all tests (SPSS v. 13.00.05, Systat Software 2009, San Jose, CA). In all cases, we used $p < 0.05$ as the significance level.

RESULTS

Black Mustard Cover

Restoration treatments significantly altered black mustard cover (Figure 1; one-way chi-square; $X^2 = 46.7$, df = 2, $p < 0.001$). Specifically, we found that herbicide (pairwise chi-square; $X^2 = 28.5$, df = 1, $p < 0.001$) as well as mowing (pairwise chi-square; $X^2 = 26.8$, df = 1, $p < 0.001$) significantly reduced mustard cover compared to controls.. In addition, the effectiveness of the treatments depended on the site (two-way chi-square; $X^2 = 22.3$, df = 2, $p < 0.001$). At UCI, the mowing treatment reduced black mustard cover significantly more than the herbicide treatment (pairwise chi-square; $X^2 = 19.6$, df = 1, $p < 0.001$). At Crystal Cove, the mowing and herbicide treatments did not significantly differ from one another in effectiveness (pairwise chi-square; $X^2 = 3.5$, df = 1, $p = 0.06$). Finally, there was no significant direct effect of site on black mustard cover (one-way chi-square; $X^2 = 2.1$, df = 1, $p = 0.145$).

AM Abundance

Restoration also significantly altered AM hyphal lengths in field soil (Figure 2; ANOVA, $p = 0.021$; Table S1). Specifically, herbicide increased AM hyphal length significantly compared to controls ($p = 0.037$). The effect of herbicide depended on field site, as indicated by a significant interaction between site and treatment ($p = 0.013$). The herbicide effect was evident in the UCI site (UCI herbicide vs UCI control: $p = 0.022$), but not at Crystal Cove (Crystal Cove herbicide versus Crystal Cove control: $p = 1.000$). Moreover, mowing did not significantly alter AM hyphal lengths ($p = 0.348$). Finally, we found a significant site effect—AM hyphal lengths were higher at UCI than Crystal Cove ($p = 0.019$).

Native Cover

Restoration treatments did not improve native plant abundance, given that there was no significant treatment effect on percentage native cover (Figure 3; one-way chi-square; $X^2 = 3.07$, df = 2, $p = 0.215$). Likewise, treatment effects did not vary between the two sites (two-way chi-square; $X^2 = 1.18$, df = 2, $p = 0.554$). Native cover was significantly higher at Crystal Cove (15.9% ± 5.8%, mean ±SE) than UCI (2.2% ±0.9%)(one-way chi-square; $X^2 = 10.4$, $p < 0.001$).

DISCUSSION

We found that benefits of invasive plant management can influence soil biota—AM fungi can become more abundant, depending on the method used by practitioners. In particular, we applied the herbicide glyphosate annually during early spring, when

invasive black mustard had germinated but native plants remained dormant (Bell et al. 2016). This approach strongly reduced black mustard cover (Figure 1). Since glyphosate kills the whole plant, decreases in black mustard cover were likely accompanied by declines in live black mustard roots and, accordingly, their production of glucosinolates. In one of our sites, AM fungal abundance increased significantly in herbicide-treated plots (Figure 2), perhaps because they were no longer inhibited by the glucosinolates (Schreiner and Koide 1993, Raybould and Moyes 2001, Lankau and Strauss 2007). Even so, native plant cover did not increase significantly in those herbicide-treated plots. Our results suggest that in some cases, carefully timed glyphosate applications could effectively reduce invasive plant cover and improve AM abundance. Although we speculate that native plant communities might recover if given more time, we did not detect any clear increase in native plant growth after our four annual restoration treatments. In addition, improvement in AM abundance with herbicide was evident in only one of the two sites, so restoration results may vary among systems.

The glyphosate treatment did not seem to inhibit AM fungi (Figure 2), which is contrary to other studies that have documented decreases in AM abundance and activity (Druille et al. 2013a, Druille et al. 2013b, Zaller et al. 2014, Druille et al. 2015). In the previous studies, glyphosate was applied directly to AM host plants in the field (Druille et al. 2015), or to AM host plants or soil in the greenhouse (Druille et al. 2013a, Druille et al. 2013b, Zaller et al. 2014). Druille et al. (2013b) reported that negative effects of glyphosate on AM host plants contributed to declines in AM fungi, potentially because glyphosate elicited carbon stress on the host plants.

Our study contrasts with these, because we were applying glyphosate to a non-mycorrhizal plant (not an AM host plant). In addition, we timed our applications to avoid exposing active AM host plants to the herbicide. We cannot rule out the possibility that glyphosate application in the field negatively affected AM fungi, or native AM host plants, to some extent. Nevertheless, AM abundance in the field either increased (at UCI) or did not change significantly (at Crystal Cove), so it appears that any negative effects were offset by positive effects from the removal of black mustard.

Others have found that applications of glyphosate can reduce invasive plants without harming surrounding native plants (Nuzzo 1996, Carlson and Gorchov 2004). Previous studies have reported that glyphosate effectiveness depends on its concentration (Nuzzo 1996). Specifically, concentrations of 0.5% were less effective at reducing mustard cover, while concentrations of 1% and 2% reduced the cover of *Alliaria*, an invasive mustard, in a mesic upland forest (Nuzzo 1996). Nuzzo (1996) reported that none of the glyphosate concentrations (0.5–2%) noticeably affected native plant species, which were dormant during the glyphosate applications in these forested ecosystems. Likewise, our study found that a single annual application of 1.68 kg/ha glyphosate did not significantly reduce native plant cover. Unfortunately, native plant cover did not significantly increase either, even though herbicide decreased black mustard cover in both sites and increased AM abundance at the UCI site. Perhaps native plant growth was limited by some other environmental condition, such as low water availability.

The timing of herbicide application can influence its effects on native plants (Crone et al. 2009). For example, when glyphosate is applied several times throughout the growing season, the biomass and density of native plants can decline (Laufenberg et

al. 2005). Loss of native forb cover also occurred when another herbicide, picloram, was added to a shrubland-grassland while the natives were active (Skurski et al. 2013). We urge caution on applying herbicides in other ecosystems where invasives and natives have similar phenologies (Steers and Allen 2010).

Unlike the herbicide treatment, mowing did not improve AM abundance (Figure 3), even though it decreased black mustard cover (Figure 1). Our mowing treatment removed black mustard shoots but left its roots intact. It is possible that the roots continued releasing organic compounds, which might have limited potential benefits of black mustard removal for AM fungi. Our findings are not consistent with those of Binet et al. (2013), who found that mowing increased AM abundance in sub-alpine grasslands. Perhaps the discrepancy is due to allelopathic effects of the black mustard roots in our study system. In addition, we found mowing had no effect on native plant abundance, possibly because thatch removal could expose soil surfaces to wind erosion and solar radiation. Both of these factors may increase water evaporation from mowed sites, which could reduce the viability of germinating seedlings and disrupt fragile hyphal networks. Altogether, our results suggest that mowing can effectively remove invasive black mustard, but does not provide corresponding benefits to either AM fungi or native plant communities.

This study does have some caveats. For example, herbicide effects were not consistent—sites differed significantly from each other in the response of AM abundance to glyphosate. Although herbicide significantly increased AM abundance at UCI, it had no significant effect at Crystal Cove. Herbicide effects may have been mediated by edaphic or climatic characteristics that differed between the sites. For example, clay-loam

soils at Crystal Cove have more loam and less clay than the clay-loam soils at UCI. Moreover, the Crystal Cove site is closer to the coast, and experiences more humidity and coastal fog than the UCI site. Additionally, there were only two replicate plots at UCI, although we were still able to detect significant changes in black mustard cover and AM abundance in that site.

In summary, we have found that a strategically-timed herbicide treatment can deter black mustard invasion and help re-establish the AM community in certain ecosystems. In contrast, mowing was less beneficial for AM fungi, but equally effective at reducing black mustard invasion. However, our treatment plots remained inhospitable to native plants. We speculate this may be due to low water availability inhibiting native plant establishment. Nevertheless, our findings support the idea that invasive species control methods can extend beyond the plant community to affect soil biota. Mycorrhizal fungi can become more abundant when invasive black mustard is removed, depending on the method used by practitioners. An explicit consideration of AM fungi may help us to refine ecological restoration practices, in order to take advantage of these naturally-occurring providers of ecosystem services.

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TABLES

Table 2.1 Native plant species detected in study.

Native plant species	**Functional group**	**Annual/perennial**
Artemisia dracunculus	forb	perennial
Baccharis pilularis	shrub	perennial
Centromadia parryi	forb	annual
Deinandra fasciculata	forb	perennial
Eriogonum fasciculatum	shrub	perennial
Encelia californica	shrub	perennial
Rumex salicifolius	forb	perennial
Solanum douglasii	forb	perennial

Table 2.2 ANOVA table for AM hyphal length

Source	**Type III SS**	**dF**	**Mean Squares**	**F-ratio**	**p-value**
site	0.175	1	0.175	12.57	0.004
treatment	0.150	2	0.075	5.40	0.021
site * treatment	0.179	2	0.089	6.42	0.013
error	0.167	12	0.014		

FIGURES

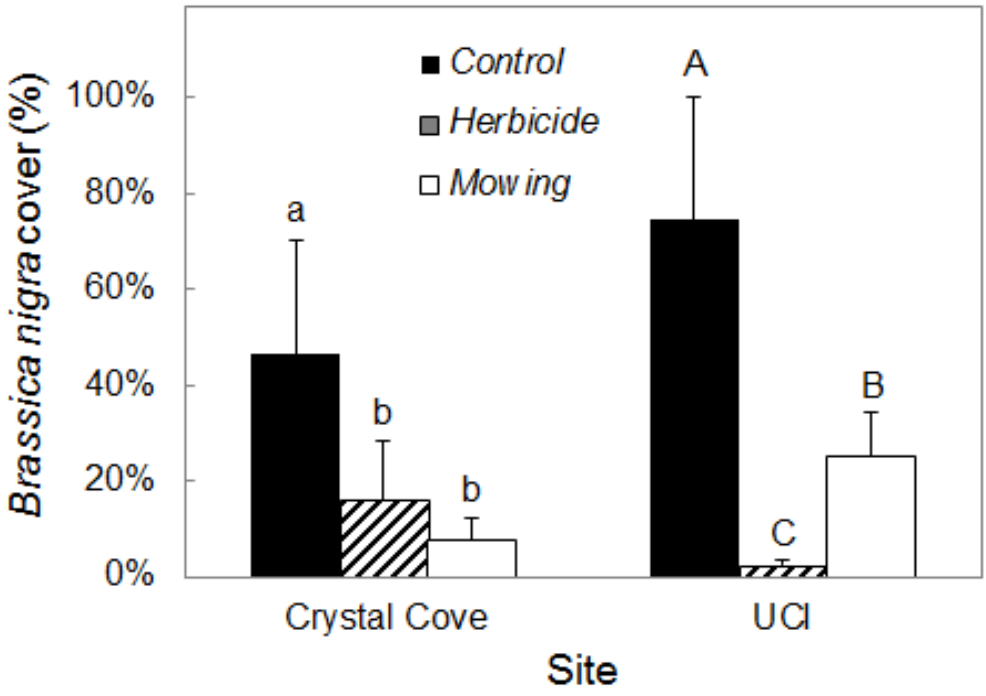

Figure 2.1. Cover of *Brassica nigra* (black mustard) plants, on a ground area basis. Mowing treatments are indicated by white bars, herbicide treatments are with grey bars, and bars filled in black are untreated controls. Treatment significantly affected black mustard cover ($p < 0.001$). Percent cover was calculated from presence/absence data from 84 random locations within each plot. Bars are means +1SE of 4 (Crystal Cove) or 2 (UCI) plots. Treatments with different letters are significantly different from one another within sites ($p < 0.05$).

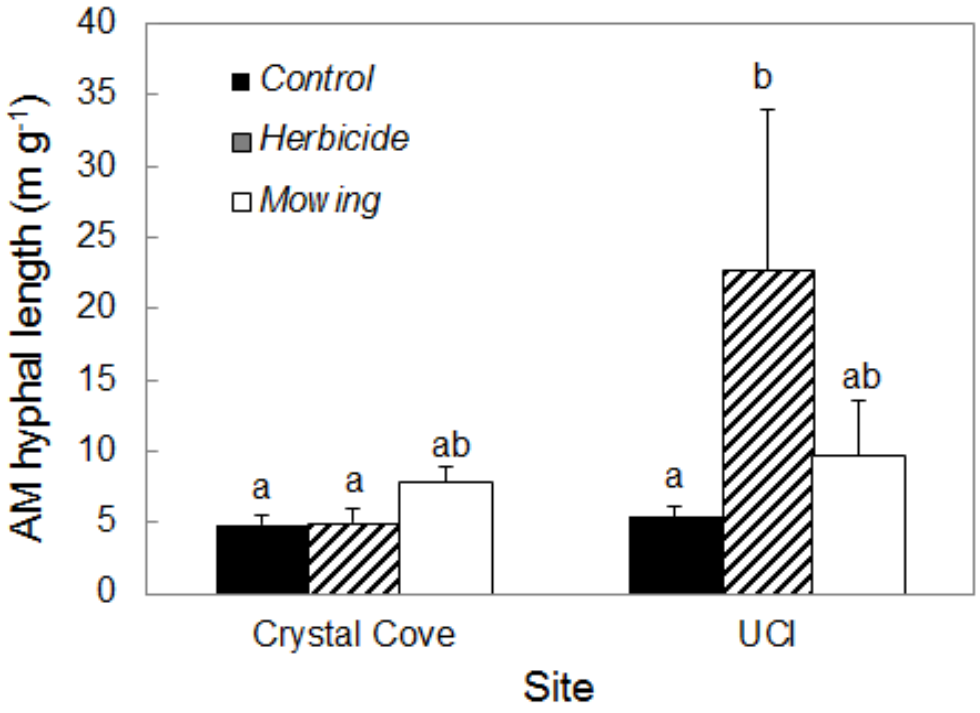

Figure 2.2. Arbuscular mycorrhizal abundance (as AM hyphal lengths) in soil collected from restoration treatment plots. Restoration treatments in white were mowed, treatments in grey were treated with herbicide, and treatments filled in black were untreated controls. There was a significant treatment effect ($p = 0.021$), site effect ($p = 0.019$), and site*treatment interaction ($p = 0.031$). Treatments with different letters are significantly different from one another ($p < 0.05$). Bars are means +1SE of 4 (Crystal Cove) or 2 (UCI) plots.

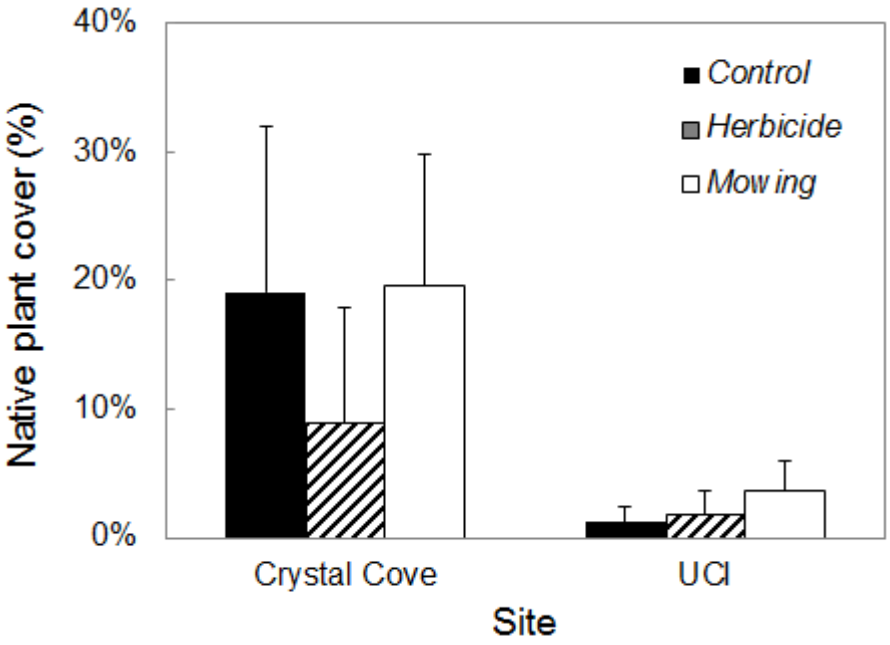

Figure 2.3. Cover of native plants, on a ground area basis. Mowing treatments are white, herbicide treatments are grey, and untreated controls are black. Native plant cover did not differ significantly between treatments ($p = 0.215$). Percent cover was calculated from presence/absence data from 84 random locations within each plot. Bars are means +1SE of 4 (Crystal Cove) or 2 (UCI) plots.

CHAPTER 3: Sources of inocula influence mycorrhizal colonization of plants in restoration projects: A meta-analysis

INTRODUCTION

Individual studies have indicated that mycorrhizal fungi can improve the success of native plant establishment in restored ecosystems (Stahl et al. 1988, Sylvia et al. 1993, Allen et al. 2003a, Graham et al. 2013). Indeed, mycorrhizal fungi provide nutrients to plants, usually leading to increased photosynthetic rates and improved plant productivity (Mosse 1957, Allen et al. 1981, McGonigle and Fitter 1988, Lekberg and Koide 2005, Hoeksema et al. 2010). Since other inhabitants rely on vegetation for food and cover, restoration of mycorrhizal fungi may have far-reaching benefits within the ecosystem. Nevertheless, little is known about the extent to which mycorrhizal fungi can be directly manipulated in restoration projects, and which sources of mycorrhizal inocula are most effective.

A number of human activities can reduce the abundance of mycorrhizal fungi in degraded ecosystems (Allen and MacMahon 1985, McGonigle and Miller 1996, Boddington and Dodd 2000, Egerton-Warburton and Allen 2000, Lekberg and Koide 2005, Sheng et al. 2012, Kohl et al. 2014). For instance, soil erosion, excessive irrigation, and agriculture practices can diminish the viability of fungal spores due to increased exposure to solar radiation, salinity, or fungicides (Hirrell and Gerdemann 1980, Saif 1981, Trappe 1981, Hayman 1982, Juniper and Abbott 2006, Tian et al. 2009, Homma et al. 2012). In addition, overgrazing can compact soils and reduce soil pore sizes, inhibiting mycorrhizal activities. Moreover, reduced vegetative cover and exotic plant invasions can alter the degree to which mycorrhizal fungi are supported by their host plants (Ehrenfeld et al. 2005, Wolfe et al. 2005, Palenzuela and Barea). Furthermore, anthropogenic introductions of salt, nitrogen, or mine tailings can often have toxic effects on mycorrhizal fungi

(Juwarkar and Jambhulkar 2008, de Souza et al. 2013, Estrada et al. 2013). Thus, direct intervention may be required to restore the abundance and function of mycorrhizal fungi in degraded ecosystems (Thrall et al. 2005, Bozzolo and Lipson 2013).

A relatively small portion of restoration projects have attempted to manipulate mycorrhizal fungi directly (Sylvia 1989, Greipsson 2010). Those who have addressed this issue typically apply inoculum (i.e., material containing spores or hyphal fragments) to soil in the field or to growth media in nursery pots prior to out-planting. The rationale for this practice is that the addition of mycorrhizal propagules should improve the likelihood that roots will encounter mycorrhizal spores or hyphae and become colonized by the fungus (Christensen and Allen 1980). Generally, plants that are more extensively colonized by arbuscular mycorrhizal fungi tend to grow better (Treseder 2013). In a recent meta-analysis, (Pineiro et al. 2013) reported that mycorrhizal inoculation was particularly effective in improving the survival and growth of planted seedlings in aridlands. Currently, it is unclear whether this technique is broadly successful across a range of ecosystem types.

Indeed, additions of mycorrhizal inoculum need not necessarily improve mycorrhizal colonization of plant roots in a given ecosystem. In some cases, mycorrhizal inoculation may have little effect on colonization because adequate inoculum is already naturally present in the ecosystem. The availability of mycorrhizal propagules is only one determinant of mycorrhizal abundance—a number of other factors may inhibit mycorrhizal fungi in ecosystems marked for restoration (Skujins & Allen 1986). For instance, harsh environmental conditions in degraded landscapes could reduce the viability of inoculum in the soil, or could inhibit the extension of hyphae from roots of nursery-inoculated plants (Plett et al. 2014, van der Heijden et al. 2015).

Thus, it is worth examining whether enhanced root colonization is sustained over the long-term (i.e., months to years) in field-established plants.

In addition, the mycorrhizal species represented in a given inoculum may not always be beneficial for the revegetated plant community. In fact, many mycorrhizal species may exhibit host preferences, host selectivity, or some degree of host specificity (Helgason et al. 1998, Smith and Read 2008, Sanchez-Castro et al. 2012, van der Heijden et al. 2015), which may not allow them to form relationships with certain restored plants. Even species of arbuscular mycorrhizal fungi—which are thought to be relative generalists—can vary in the degree to which they colonize plant roots (Powell et al. 2009) and in their effects on plant growth (Hoeksema et al. 2010). Mycorrhizal fungi from neighboring reference ecosystems (i.e., ecosystems that exhibit characteristics intended of the restored ecosystem) may be better-suited for the local environment. Indeed, in a recent review of restoration studies from semiarid ecosystems, (Barea et al. 2011) noted that inocula derived from exotic mycorrhizal fungi may not be as effective as inocula from indigenous fungi. Common sources of mycorrhizal inocula for restoration work include: commercially-available inocula, whole inocula derived from soil or roots collected from reference ecosystems, or species-specific inocula isolated in the laboratory. If we can identify sources of inocula that best-improve mycorrhizal abundance, this information can be used by restoration ecologists to develop best practices.

Toward this end, we performed a meta-analysis of published restoration studies that examined effects of mycorrhizal inoculation on mycorrhizal establishment (as percent root length colonized, PRLC) and plant growth under field conditions. We tested the hypotheses that additions of mycorrhizal inocula would result in sustained increases in PRLC (Hypothesis 1) and improved plant growth (Hypothesis 2) in the field, and that whole inocula from neighboring

reference ecosystems would elicit larger increases in PRLC than would commercial inocula (Hypothesis 3).

MATERIALS AND METHODS

We surveyed articles published in the literature and found 28 manipulative field-based restoration trials from 22 publications that addressed the influence of mycorrhizal fungal inoculation on percent colonization of plant roots. We used the Google Scholar (scholar.google.com) and Web of Science (webofknowledge.com) search engines, and also directly searched the archives of the Restoration Ecology journal (link.springer.com/journal/572). Our search terms were (restor* and mycorrhiz*), (restor* and inocul*), or (restor* and fung*). We used the following "decision rules" to select trials: the projects must have (1) used active ecological restoration of a degraded or constructed ecosystem, (2) incorporated an inoculated treatment and an uninoculated control, and (3) measured PRLC on (4) field-grown plants. We included trials from a variety of ecosystem types, geographic locations, and disturbance types. The landscapes in the selected trials were degraded primarily by human activities (Table 1).

In each trial, mycorrhizal colonization was directly manipulated via the addition of mycorrhizal inoculum as an active restoration technique. The fungal inoculum was sourced from reference ecosystems, commercial sources, or specific fungal isolates (Table 1). Commercial sources included mycorrhizal fungi mixed with unspecified/proprietary granular materials (e.g., Nutri-Link from Schenck & Smith, Native Plants Inc.) and pre-inoculated seedlings that were prepared by commercial nurseries using proprietary methods (Sylvia et al. 1993, de Aragón et al. 2012). Some of the species-specific inocula were prepared from soil originating from the

experimental site, mycelial cultures obtained from curated collections, or sporocarps originating from either the experimental site or other ecosystems (Rincon et al. 2007, Alguacil et al. 2011). Some mycorrhizal species were selected based on their desired ecological traits, such as production of abundant fruitbodies or facilitation of plant establishment. Every selected trial included an inoculated treatment group compared with an uninoculated control group. Fungal inoculum was either applied directly to soils in the field study site, or to plants in the greenhouse prior to out-planting in the field site.

Plants grew in the field for 4 months (Sylvia et al. 1993) to 54 months (de Aragón et al. 2012). Next, the plants were harvested and roots were analyzed for mycorrhizal colonization. In all cases, microscopy was used to evaluate the mycorrhizal structures in plant roots. Four trials quantified ectomycorrhizal fungi on unstained roots. The remainder examined arbuscular mycorrhizal fungi, following staining with trypan blue. Likewise, all of the trials examined fungal structures and measured the percent root length colonized by using the gridline intersect method (Phillips and Hayman 1970, Ambler and Young 1977, Giovanetti and Mosse 1980, McGonigle et al. 1990, Brundrett et al. 1994).

We excluded studies that lacked a direct field component or measured mycorrhizal abundance using spore density or other metrics besides PRLC. To maintain independence of trials, in the case of longitudinal studies that used several time points to measure PRLC, we included only the final PRLC measurement recorded (i.e., representing the longest duration of time). If a study reported multiple sets of results (e.g., geographic location or inoculum type) in which an independent untreated control group was compared with an inoculated treatment group, then each system was designated as a different trial (Sylvia et al. 1993, Al Agely and Sylvia 2008).

For each trial, we obtained the mean PRLCs and numbers of replicates (n) for the inoculated treatment and uninoculated controls. We used these data along with reports of standard error, standard deviation, or summary statistics to calculate a product-moment correlation (r) for each study as in Rosenthal (1991). We then calculated Fisher's z-transform as an effect size for each study, using the formula:

$$Fisher's\ z_{PRLC} = \frac{1}{2} ln \left(\frac{1 + r}{1 - r} \right)$$

In addition, the variance of z was calculated as:

$$v_z = \left(\frac{1}{n\ -\ 3} \right)$$

For all but one trial, the investigators also measured some aspect of plant performance, usually as shoot dry mass or seedling height (Table 1). We calculated Fisher's z_{plant} and its variance from these data as described for Fisher's z_{PRLC}, above.

To test Hypothesis 1, we used a random-effects model to estimate a cumulative Fisher's z_{PRLC} of all 28 trials (Rosenberg et al. 2007). Each trial was weighted by the reciprocal of the variance of z (v_z). In addition, 95% confidence intervals were calculated via bias-corrected bootstrapping with 999 iterations (Rosenberg et al. 2007). We compared the cumulative Fisher's z_{PRLC} against a mean value of zero. Hypothesis 1 would be supported if the cumulative Fisher's z_{PRLC} were significantly greater than zero. Likewise, Hypothesis 2 would be supported if the cumulative Fisher's z_{plant} of the 27 trials were significantly greater than zero.

To test Hypothesis 3, and check for other aspects of mycorrhizal restoration methods that might influence outcomes, we performed a series of categorical model meta-analyses on grouped data (Rosenberg et al. 2007). Specifically, for Hypothesis 3, we tested for differences in

cumulative Fisher's z between trials that used various inoculum sources (e.g., whole inocula from reference ecosystem, single species inoculum, or commercial inoculum). Hypothesis 3 would be supported if the cumulative Fisher's z were significantly higher in trials that used whole inocula from references ecosystems than in those that used commercial inocula. We also tested for significant differences among trials that inoculated seedlings in the nursery (followed by outplanting) versus those that applied inoculum in the field. Likewise, we compared studies that measured AM versus ECM colonization of plant roots, and checked for differences among ecosystem types.

Finally, we performed two continuous model meta-analyses (Rosenberg et al. 2007) to examine whether inoculum effects decreased over time under field conditions. For each test, the continuous variable was the amount of time that inoculated plants grew in the field before being assayed for PRLC or plant growth (i.e., "duration in field"). Fisher's z_{PRLC} was the effect size for one test; Fisher's z_{plant}, the other.

We checked for "file-drawer" biases, by which failure to publish null effects of inoculation would influence our results. We used two tests: Kendall's Tau test for rank correlations between effect size and sample size, and Orwin's fail-safe N test. We used MetaWin 2.0 for all analyses, and effects were considered significant when $P < 0.05$.

RESULTS

Characteristics of selected studies

The selected trials were conducted on landscapes degraded primarily by anthropogenic activities such as agriculture, logging, construction, desertification, grazing, and mining (Table 1). The majority were based in the Northern Hemisphere, with 15 studies from North America

and nine from Western Europe. The remaining studies were located in Morocco, Indonesia, and South America. Coastal dunes, shrublands, and temperate forests were the dominant ecosystem types, followed by tropical forests, temperate grasslands, and tropical savanna. Inoculation of seedlings in the nursery, followed by outplanting in the field site, was more common than the application of inoculum directly to soil in the field.

General responses to inoculation

Across all 28 trials, inoculation with mycorrhizal fungi increased PRLC, as indicated by a cumulative Fisher's z_{PRLC} of 0.65 (0.40–0.94, 95% confidence interval), which was significantly greater than the null value of zero (Fig. 1, $P < 0.001$). The improvement in mycorrhizal abundance was accompanied by a significant increase in plant growth in the inoculated treatments, with a cumulative Fisher's z_{plant} of 0.57 (0.36–0.91, 95% CI) across all 27 trials that measured plant responses (Fig. 2, $P < 0.001$). These results supported Hypotheses 1 and 2, respectively. Moreover, the inoculation effects did not notably decline with longer exposure to field conditions (Fig. 3). Specifically, the effects of inoculation on PRLC ($Q = 0.332$, $P = 0.498$) and plant growth ($Q = 0.695$, $P = 0.565$) did not vary significantly with the duration of the field component.

The meta-analysis did not appear to be particularly sensitive to publication bias. Specifically, Kendall's Tau tests were not significant for mycorrhizal colonization ($\tau = 0.009$, $P = 0.949$) or plant responses ($\tau = 0.099$, $P = 0.469$). Moreover, fail-safe tests indicated that 76 mycorrhizal colonization trials with null results would need to be added to render the inoculation effect non-significant. For plant responses, 42 null-results trials would need to be added.

Variation among sources of inocula

Sources of inocula elicited significantly different effects on PRLC (Fig. 1, P = 0.047). In particular, inocula from reference ecosystems and single species yielded higher increases in PRLC than did inoculum from commercial sources (Fig. 1). The pairwise differences between inocula from reference ecosystems and commercial sources supported Hypothesis 3. We note that values of Fisher's z_{plant} associated with the different inocula sources tended to display the same pattern as for Fisher's z_{PRLC}, but in this case, differences among sources were not significant (Fig. 2, P = 0.317).

Other factors

We observed no significant differences in Fisher's z_{PRLC} between nursery-inoculated versus field-inoculated trials (Fig. 1, P = 0.573), AM versus ECM fungi (H = 0.369, P = 0.458), or ecosystem types (P = 0.372). Moreover, Fisher's z_{plant} did not differ significantly between inoculation methods (Fig. 2, P = 0.729), mycorrhizal types (P = 0.114), or ecosystem types (P = 0.153).

DISCUSSION

Our findings suggest that restoration ecologists can intentionally increase the abundance of mycorrhizal fungi in degraded ecosystems, and thus improve the establishment of native plants. Moreover, these benefits can last up to several years. Specifically, mycorrhizal colonization of plant roots in field-based restoration projects significantly increased, on average, when mycorrhizal inocula was added (Fig. 1). At the same time, plant performance in the field significantly improved (Fig. 2). Neither effect declined significantly with time (Fig. 3). These responses are consistent with our understanding of the ecology of mycorrhizal fungi—they are

important plant mutualists (Allen et al. 2003b, Smith and Read 2008, Hoeksema et al. 2010) that are sensitive to anthropogenic disturbance (Cudlin et al. 2007, Compant et al. 2010, Pickles et al. 2012, Mohan et al. 2014) and may require restoration in their own right.

Moreover, certain sources of inocula were more effective than others. When fungal inoculum from a reference ecosystem or a single fungal taxon was used to inoculate field restoration projects, PRLC increased significantly more than when commercial inoculum was used (Fig. 1). Likewise, Sylvia et al. (1993) examined the effects of different inoculum types in Florida dune restorations and found that native mycorrhizal inocula consistently yielded greater mycorrhizal colonization than commercial inocula. Higher root colonization from reference inocula could result from complementary interactions between the fungi and host plants that have developed over time under comparable conditions. In contrast, when a commercial source of inoculum is used, inoculation may shift the composition of the mycorrhizal community away from specialist native mycorrhizal fungi towards generalist 'weedy' mycorrhizal fungi that might be less effective mutualists for native plants (Barea et al. 2011). Indeed, Koch et al. (2011) found that incorporating exotic mycorrhizal fungi into a degraded site substantially changed mycorrhizal communities—even more than plant invasions did. Mycorrhizal community composition may influence restoration outcomes, because mycorrhizal species differ in their ability to form relationships with a given plant species, and in their responses to environmental conditions (van der Heidjin 2002, Klironomos 2003, Morris et al. 2007, Hoeksema et al. 2010). The difference observed here in effectiveness of inoculum sources is consistent with this notion.

Researchers have long investigated the role of mycorrhizal fungi in ecological restoration (Ramos-Zapata et al. 2006, Siguenza et al. 2006, van der Wal et al. 2006, White et al. 2008, Vargas et al. 2010). For example, Skujins and Allen (1986) recommended facilitating

mycorrhizal growth in degraded sites via inoculation and soil organic retention. Recently, (Pineiro et al. 2013) synthesized data from restoration projects in degraded drylands to compare effectiveness of various restoration techniques. They reported that inoculation with mycorrhizal fungi was generally better than tree shelters, organic amendments, and hydrogels in improving the growth and survival of seedlings. Likewise, Barea et al. (2011) reviewed the use of mycorrhizal inoculation in revegetation projects on degraded semiarid lands in Southeast Spain, finding that inoculation improved both plant development and soil quality. Barea et al. (2011) also compared native inoculum from reference ecosystems versus exotic commercial inoculum and found that in dry environments, native, drought adapted mycorrhizal fungi improve plant performance more than non-native mycorrhizal fungi. Our meta-analysis extends these observations beyond drylands, and suggests that inoculation could improve colonization and plant growth in a variety of ecosystems.

Similar findings have been documented in studies that did not involve restoration. For example, Lekberg and Koide (2005) conducted a meta-analysis on agricultural systems, and reported that mycorrhizal colonization and plant biomass was augmented by inoculation. Moreover, Hoeksema et al. (2010) conducted a broad meta-analysis of the effects of mycorrhizal inoculation on plant growth in studies based in laboratories, agricultural fields, plantations, and natural ecosystems (including one restoration project). They observed increases in plant growth in response to inoculation in the field as well as the laboratory. None of these other meta-analyses compared inoculation methods in the nursery to those in the field.

In our meta-analysis, inoculation of mycorrhizal fungi increased plant performance, on average (Fig. 2). Plant responses to inoculation treatments largely mirrored those of mycorrhizal colonization. If inoculation increases PRLC, then this in turn increases the surface area of

mycorrhizal fungi interacting with plant roots and accessing nutrients in the soil environment. Plants with greater PRLC would then benefit from receiving more nutrients from mycorrhizal fungi than would uninoculated plants (Fitter 1985, Read 1999, Varma 1999, Allen et al. 2003b). In fact, Barea et al. (2011) demonstrated that inoculation increases outplanting performance, survival, and plant biomass in restoration projects in semiarid lands, ostensibly because mycorrhizal fungi improve plant resistance to the drought stress, nutrient deficiency, and soil degradation that are common in degraded ecosystems.

Our findings can be useful in developing best practices in restoration ecology. Intentional inoculation could improve the growth and establishment of a mycorrhizal community in restored ecosystems, which may benefit aboveground vegetation communities. In addition, using inoculum from a reference ecosystem or a species-specific inoculum may increase PRLC more than a commercial inoculum. The use of inocula from reference ecosystems could also be economically advantageous for practitioners who otherwise might purchase commercial inocula. In large-scale projects, the cost of commerical inocula would not be trivial. There was no significant difference between PRLC in species-specific inoculum and from reference ecosystems, yet it is more technically challenging and time-consuming to isolate mycorrhizal species than it is to obtain inoculum from a reference ecosystem.

When obtaining inocula from undisturbed sites, practices that might harm reference ecosystems should be avoided. To reduce disturbance, sourcing small volumes of soil from reference sites to inoculate plants in the greenhouse prior to out-planting in the field may be preferable to transferring large volumes of native soil (Cairns 1995). Furthermore, sourcing inoculum from edges of reference ecosystems could decrease disturbance in untrammeled sites (Mitsch and Jørgensen 2004). Sourcing inocula from restored sites with similar historical

disturbances could be advantageous, because fungal propagules from these restored sites may be adapted to the special conditions associated with a particular disturbance (Greipsson 2010, Orlowska et al. 2011). In cases where reference sites are slated for development, topsoil containing fungal propagules could be salvaged from the reference site prior to construction or development (Rowe et al. 2007). Nevertheless, it is worth considering that after several months, soil stockpiling may reduce mycorrhizal abundance and compromise the effectiveness of this inoculum source (Galatowitsch 2012). The most appropriate inoculum choice depends on several factors, but altogether, it may be more practical and economically feasible for restoration ecologists to integrate reference inoculum into their restoration protocols, instead of species-specific or commercial inoculum.

The number of trials represented in this meta-analysis is somewhat small, because we restricted our selection to restoration projects that assessed PRLC. Nevertheless, we were able to detect significant effects of inoculation across trials. Furthermore, inoculation effects were positive in the majority of trials—only four of 28 trials reported a decline in PRLC, and three of 27 in plant growth. Nevertheless, we note that even though a variety of ecosystem types were represented, the trials were mostly based in temperate ecosystems in the Northern Hemisphere. Additional research in tropical ecosystems and the Southern Hemisphere would be valuable.

While mycorrhizal fungi are just one component of a broader belowground community, they are the only component of the microbial community we examined in this meta-analysis. This focus is deliberate; O'Neill et al. (1991) posited that mycorrhizal fungi are ‘keystone mutualists’ in terrestrial ecosystems, and therefore may exert a disproportionate influence on other soil microbes on the site. If mycorrhizal fungi affect community structure and ecosystem processes, and thus restoration outcomes, then they may influence other members of the soil

microbial community. Moreover, mycorrhizal inoculation may increase plant cover through added plant biomass, which could lead to increased protection of exposed soil surfaces from solar radiation and other harsh environmental conditions. This added plant biomass would also provide substrate for decomposer microbes that may provide additional benefits to restored ecosystems, such as erosion control or increased soil organic matter. Future studies exploring the role of mycorrhizal inoculation in influencing the total microbial community and ecosystem processes at restoration sites would clarify the direct and indirect roles of these fungi (Brunson et al. 2010, Kulmatiski 2011, Binet et al. 2013).

In conclusion, this meta-analysis indicates that mycorrhizal fungi can be directly manipulated in many restoration sites via inoculation. In turn, inoculation generally improved restoration success by increasing plant performance. Moreover, sources of inocula varied in their effects on mycorrhizal colonization. In particular, the use of inoculum from reference ecosystems may be particularly effective and practical, compared to species-specific and commercial inocula. Land managers may wish to consider incorporating mycorrhizal fungi in their restoration efforts to better facilitate the establishment of below- and aboveground components of ecosystems.

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TABLE

Table 3.1. Studies included in meta-analysis.

Trial	Location	Ecosystem type	Disturbance type	Inoculum type	Inoculation method	Mycorrhizal type	Duration in field (months)	Fisher's z_{PRLC}	Var(Z_{PRLC})	Plant parameter	Fisher's z_{plant}	Var (Z_{plant})
Al Agely & Sylvia 2008	29°53′N, 81°17′W	Coastal dunes	-	from reference ecosystem	Inoculation in nursery	AM	24	1.00	0.05	shoot dry mass	1.13	0.05
Al Agely & Sylvia 2008	29° 39′N, 84°52′W	Coastal dunes	-	from reference ecosystem	Inoculation in nursery	AM	24	0.78	0.05	shoot dry mass	1.66	0.05
Alguacil et al. 2011	38°12′N, 1°13′W	Shrubland	agriculture	single species	Inoculation in field	AM	14	0.01	0.11	shoot dry mass	1.02	0.11
Allen et al. 2005	21°12′N, 87°10′W	Tropical forest	fire	from reference ecosystem	Inoculation in nursery	AM	26	0.00	0.14	seedling height	0.16	0.14
Caravaca et al. 2003	38°23′N, 1°10′ W	Shrubland	desertification	single species	Inoculation in nursery	AM	18	2.65	0.20	shoot dry mass	3.45	0.20
Cook et al. 2011	47°58′N, 123°35′W	Temperate forest	dam	from reference ecosystem	Inoculation in field	AM and EM	20	0.00 (AM)	0.33 (AM)	shoot+root mass	-0.45	0.33
Cuenca et al. 1998	5°40′N, 61°32′W	Tropical savanna	road construction	natural inoculum not from reference ecosystem	Inoculation in field	AM	5	1.33	0.06	aboveground biomass per unit ground area	0.37	0.06
de Aragón et al. 2012	41°35'N, 1°31'E	Temperate forest	fire	commercial	Inoculation in nursery	EM	54	0.10	0.03	seedling height	0.17	0.03

de Souza et al. 2010	6°28′S, 34°55′W	Coastal dunes	mining	single species	Inoculation in field	AM	13	0.60	0.09	seedling height	0.48	0.09
Duponnois et al. 2011	31°54′N, 8°17′W,	Shrubland	desertification	from reference ecosystem	Inoculation in nursery	AM	36	0.38	0.33	seedling height	1.07	0.33
Gemma & Koske 1997	42°4′ N, 70°9′ W	Coastal dunes	grazing	from reference ecosystem	Inoculation in field	AM	11	-0.02	0.33	culms/hill	1.13	0.33
Graham et al. 2013	2°18′ S, 113°50′ E	Tropical forest	logging	single species	Inoculation in nursery	AM and EM	6	0.40 (AM & EM)	0.04 (AM & EM)	shoot dry mass	0.40	0.04
Pagano et al. 2009	15°9′ S, 43°49′ W	Tropical forest	logging	three species	Inoculation in nursery	AM and EM	16	0.66 (AM)	0.11 (AM)	-	-	-
Palenzuela et al. 2002	38°23′ S, 1°10′ W	Shrubland	desertification	single species	Inoculation in nursery	AM	8	1.42	0.20	shoot dry mass	0.28	0.20
Querejeta et al. 2003	38°23′ S, 1°10′ W	Shrubland	-	single species	Inoculation in nursery	AM	8	2.30	0.03	shoot dry mass	1.02	0.03
Requena et al. 2001	36°50'N 2°27'W	Shrubland	desertification	from reference ecosystem	Inoculation in nursery	AM	10	0.97	0.20	shoot dry mass	0.34	0.20
Richter & Stutz 2002	31°32′ N, 110°4′ W	Temperate grassland	agriculture	from reference ecosystem	Inoculation in nursery	AM	10	0.52	0.11	seedling height	0.54	0.11
Rincón et al. 2007	40°25'N 3°42'W	Temperate forest	industrial activities	single species	Inoculation in nursery	EM	24	0.48	0.14	seedling height	0.48	0.14
Roldán et al. 1996	37°59'N 1°7.8'W	Temperate forest	topsoil clearing	single species	Inoculation in nursery	EM	14	0.97	0.14	seedling height	0.05	0.01

Smith et al. 1998	45°60′ N, 93°21′ W	Temperate grassland	road construction	from reference ecosystem	Inoculation in field	AM	15	0.62	0.20	% cover	0.12	0.20
Stahl et al. 1988	43°4.5'N 107°17'W	Shrubland	mining	from reference ecosystem	Inoculation in nursery	AM	12	-0.06	0.11	plant height	0.28	0.11
Sylvia et al. 1993	25°47'N 80°7.8'W	Coastal dunes	constructed beaches	from reference ecosystem	Inoculation in nursery	AM	6	1.13	0.33	shoot dry mass	1.13	0.33
Sylvia et al. 1993	25°47'N 80°7.8'W	Coastal dunes	constructed beaches	commercial	Inoculation in nursery	AM	6	-1.13	0.33	shoot dry mass	0.06	0.33
Sylvia et al. 1993	30°19'N 81°39'W	Coastal dunes	constructed beaches	from reference ecosystem	Inoculation in nursery	AM	4	1.13	0.33	shoot dry mass	0.06	0.33
Sylvia et al. 1993	30°19'N 81°39'W	Coastal dunes	constructed beaches	commercial	Inoculation in nursery	AM	4	-0.06	0.33	shoot dry mass	-0.06	0.33
Sylvia et al. 1993	30°27'N 86°33'W	Coastal dunes	-	from reference ecosystem	Inoculation in nursery	AM	4	1.13	0.33	shoot dry mass	-0.06	0.33
Sylvia et al. 1993	30°27'N 86°33'W	Coastal dunes	-	commercial	Inoculation in nursery	AM	4	0.00	0.33	shoot dry mass	0.06	0.33
Walker 2003	38°42′ N, 119°39′ W	Temperate forest	mining	single species	Inoculation in nursery	EM	17	0.08	0.14	shoot volume	0.06	0.14

FIGURES

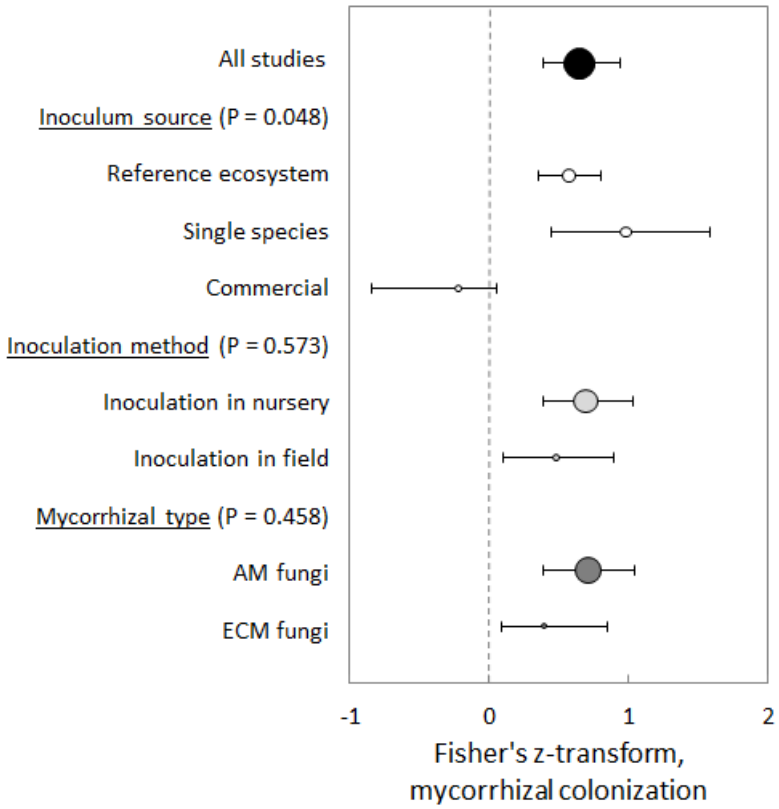

Figure 3.1. Cumulative effect sizes (as Fisher's z-transform) for changes in percent root length colonized (PRLC) by mycorrhizal fungi in response to inoculation in restoration projects. Symbols are means ±95% confidence intervals of trials. Error bars that do not overlap with zero (dashed line) indicate significant effects of inoculation on PRLC. P-values indicate significance of variation in cumulative Fisher's z among inoculum sources, inoculation methods, and mycorrhizal types. Symbol size is proportional to number of trials (4–28). AM = arbuscular mycorrhizal, ECM = ectomycorrhizal.

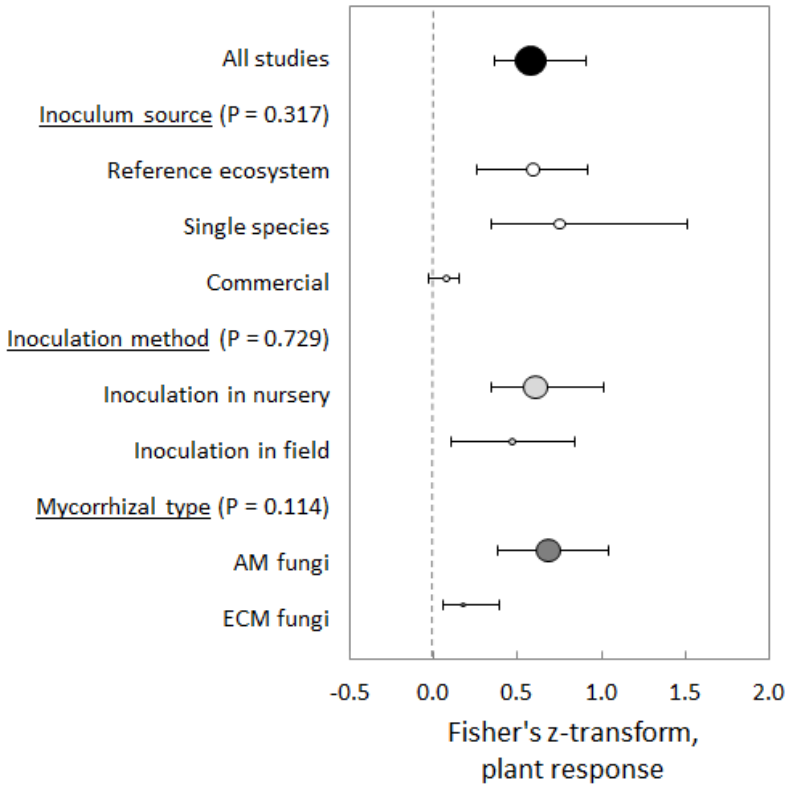

Figure 3.2. Changes in plant growth in response to inoculation by mycorrhizal fungi (as Fisher's z-transform) in restoration projects. Symbols are means ±95% confidence intervals of trials. Error bars that do not overlap with zero (dashed line) indicate significant effects of inoculation on plant growth. P-values indicate significance of variation in cumulative Fisher's z among inoculum sources, inoculation methods, and mycorrhizal types. Symbol size is proportional to number of trials (4–27). AM = arbuscular mycorrhizal, ECM = ectomycorrhizal.

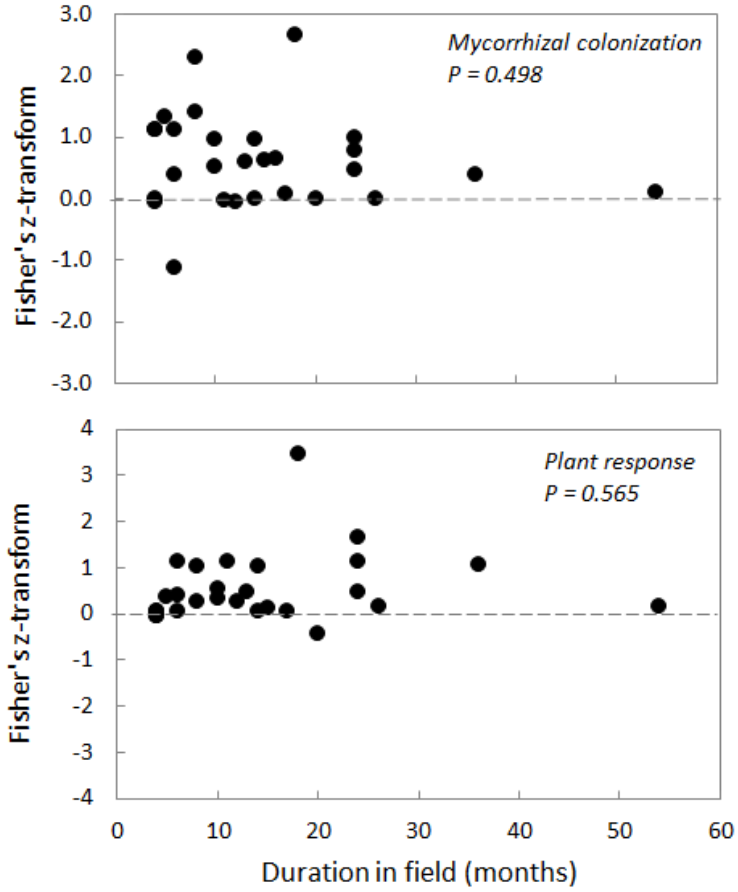

Figure 3.3. Effect sizes (as Fisher's z-transform) of inoculation on percent root length colonized (top panel) and plant growth (bottom panel) in relation to the length of time that inoculated plants were grown in the field. Neither relationship is significant. Each symbol represents one trial; all 28 (for ΔPRLC) and 27 (for plant response ratio) trials from the meta-analysis are included. Dashed reference lines indicate effect sizes associated with no change in PRLC or plant growth.