MYCORRHIZAL AND MICROBIAL INOCULATION AFFECT THE GROWTH AND SURVIVAL OF NATIVE PLANTS RAISED FOR RESTORATION

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ABSTRACT

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Production of native seedlings for field outplanting has become a common ecological restoration technique worldwide. However, the establishment of greenhouse-raised plants in the field is usually poor. Mycorrhizal fungi are symbionts that can provide survival benefits to host plants. This relationship is ubiquitous in terrestrial ecosystems and mycorrhizae are absent only under unusual circumstances, such as in a nursery greenhouse.

Nine plant species native to the highly endangered Northwest short-grass prairie and oak savanna ecosystems (*Balsamorhiza deltoidea, Castilleja levisecta, Dodecatheon hendersonii, Dodecatheon pulchellum, Festuca roemeri, Gaillardia aristata, Micranthes integrifolia, Ranunculus occidentalis,* and *Silene douglasii*) were grown for six months in sterilized medium with an arbuscular mycorrhizal fungi (AMF) inoculant cultured from local native plants, a general AMF inoculant, or in control treatments. Three microbial inoculants with AMF removed, created from a nearby site considered to be high-quality remnant prairie, a restoration site, and unsterilized potting medium, were added within each AMF treatment in a full factorial design. Seedling emergence, survival, aboveground growth, and biomass data were collected, and remaining plants were transferred to field sites for long-term monitoring.

AMF significantly enhanced the growth of five species and the survival of four, with no detectable effect on the remainder. Further, there was no significant difference between the two AMF inoculants. Field microbial wash tended to have a negative effect on seedling emergence and growth, with the high-quality site treatment most repressive. AMF and the introduced microrganisms interacted on *Festuca roemeri,* with AMF mediating the negative effect of other fungi. Surprisingly, AMF positively affected the growth of *Castilleja levisecta,* a hemiparasite, and altered the phenology of *Dodecatheon hendersonii,* delaying dormancy. These results suggest that AMF can enhance the growth and survivorship of many species, and that inoculation may lead to greater success in ecosystem restoration efforts.

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Finally, I would like to acknowledge the indigenous people of the Pacific Northwest who lived in and maintained the prairie and oak savannas for thousands of years, and whose people and culture, like their lands, have been unquantifiably damaged by invasion that continues today.

ABBREVIATIONS AND DEFINITIONS

AMF: arbuscular mycorrhizal fungi. A phylogenetic group (Glomeromycota) of fungi composed of branching hyphae that enter the roots of plants and form a symbiotic relationship in which water and nutrients are exchanged with autotrophs for carbon
AQFO: *Aquilegia formosa* Fisch. ex DC. "Western columbine"
Autotroph: Plant that photosynthesize
BADE: *Balsamorhiza deltoidea* Nutt. "Deltoid balsamroot"
CALE: *Castilleja levisecta* Greenm. "Golden paintbrush." An Endangered Species Act–listed hemiparasite that can photosynthesize but also gains nutrition by extending rootlike organs called haustoria into the belowground systems (but not cells) of other plants.
CNLM: The Center for Natural Lands Management.
DOHE: *Dodecatheon hendersonii* A. Gray. "Mosquito bills"
DOPU: *Dodecatheon pulchellum* (Raf.) Merr. "Darkthroat shootingstar"
GAAR: *Gaillardia aristata* Pursh. "Blanketflower"
Hemiparasite: a plant that can photosynthesize but often gains nutrition through feeding off the roots of other plants
Inoculant: mycorrhizal and/or microbial cultures added to soil growing medium.
JBLM: Joint Base Lewis McChord. The owner of the largest intact remnants of Pacific Northwest prairie. This large site south of Tacoma was acquired for Department of Defense use early in the 20th century due to its open landscape, and much of the area has ironically been maintained by the constant setting of fires from artillery since.
MH: mycoheterotrophic plant. A plant that does not photosynthesize for itself and instead gains nutrition by accessing carbon allocated to mycorrhizal fungi by other plants through the exploitation of mycorrhizal networks
MIIN: *Micranthes integrifolia* (Hook.) Small. "Wholeleaf saxifrage"
mycorrhiza pl. mycorrhizae: literally "fungal root." Several functional groups of mycorrhizal fungi exist, all form symbiotic relationships with plants
MN: mycorrhizal network. A condition under which multiple plants are linked by a one mycorrhizal fungus through its hyphae.
NM: non-mycorrhizal**.** An unusual state in which a plant cannot or does not form a relationship with mycorrhizal fungi.
RAOC: *Ranunculus occidentalis* Nutt. "Western buttercup"
SIDO: *Silene douglasii* Hook. "Douglas's catchfly"
WPG: Willamette Valley–Puget Trough–Georgia Basin. An ecoregion containing distinct and threatened prairies and oak savannas

INTRODUCTION

This thesis explores the possibility of using microscopic organisms to help solve some very large problems. Globalization and human exploitation of fossil fuels are causing constant and unpredictable environmental crises on earth. Among these, the loss of important natural and cultural landscapes, and a steady decline in biodiversity globally, are especially concerning, and many scientists suggest that we are in the midst of Earth's sixth great extinction event (Barnosky et al. 2011). Current human practices are not predicted to experience major positive changes in the near future and the effects of increasing CO_2 will exacerbate environmental conversion and the loss of biodiversity (Thomas et al. 2003). In the face of this dire future conservationists are working hard to mitigate changes by restoring habitats and attempting to prevent extinction.

Encouraging the re-establishment of viable wild populations of rare and native plant species through cultivation and outplanting to historical habitats is a widespread restoration technique (Machinski & Haskins 2012). Plant reintroduction attempts to mitigate loss of biodiversity and to prevent extinction by increasing native plant abundance and diversity, with a resultant preservation of species across trophic levels. The propagation of native plants for restoration is a prevalant, accepted practice that is generally unsuccessful, with very few reintroduced plants surviving more than a year or two, and even fewer establishing, flowering, or fruiting over time (Godefroid et al. 2010). Failure may be due to horticultural techniques that provide abundant nutrients but poorly emulate native environments (Haskins & Pence, 2012).

This manuscript-style thesis investigates whether inoculating growth medium with mycorrhizal fungi and other rhizosphere microorganisms may provide plants with

important traits, adaptations, and tools that will lead to greater long-term establishment in the field. The first chapter, a literature review, begins by exploring some of the problems that the practice of native plant reintroduction has experienced. It provides background on the evolution and biology of mycorrhizal symbiosis, a relationship between autotrophs and mutualistic fungi in which most terrestrial plants engage, and a more detailed analysis of the effects of arbuscular mycorrhizal fungi (AMF) on plants and ecosystems. Previous AMF inoculation research, including the formation of mycorrhizal networks (MN) between multiple plants, and emerging themes from the literature are included.

Few published studies on the use of AMF for rare and native plant propagation exist, but the compelling findings and problems of several of these are explored, as are potential AMF sources and associated costs and risks. AMF engage in complex relationships with other microorganisms, and literature related to rhizosphere interactions and their application to research methods are reviewed. Finally, the highly endangered prairie-oak savannas of the Pacific Northwest, USA, and the potential use of mycorrhizal inoculation techniques as part of the restoration strategy for this rare ecosystem is proposed.

The second chapter, an original research manuscript formatted for publication presents the results of an experiment that addresses the following questions 1) How does AMF inoculation affect the growth of greenhouse-raised seedlings? 2) Does AMF affect short-term (6 months) survival? 3) Is an AMF inoculant cultured from native soils superior to a commercially available one? 4) How will AMF inoculants interact with different soil microbial communities likely to be present in outplanting sites?

While the results found in the manuscript raise interesting basic science questions, the work was done specifically to provide information for practice. Many of the ideas in this thesis are rooted in the field of restoration ecology, and the third chapter analyzes some of the ethical and practical issues associated with this emerging science. This chapter integrates the thoughts of both philosophers and scientists to explore the changing relationship between humans and nature, important criticisms to the ideas at the foundation of restoration, and some of the problems with ecological restoration as currently practiced.

Mycorrhizal symbiosis and ecological restoration are highly complex processes that need to be understood within the context of theory. The complexities inherent to both this keystone biotic relationship, and restoration ecology itself, will undoubtedly be exacerbated by human-environmental conflict and a changing climate. A growing understanding of environmental processes, including plant-microorganism interactions and the possibilities and limitations of applied ecology, will be useful to creating positive change in the future. The native plants grown as part of this thesis have been transferred to field sites for long-term monitoring, and I hope that both these seedlings, and the ideas of this work will continue to thrive and have a positive effect on our world.

Literature Review

NATIVE PLANT REINTRODUCTION

Reintroduction of rare, native, and endangered plant species has become an important restoration tool worldwide. Success can prevent extinction, benefit species across trophic levels, and restore ecosystem functionality to degraded sites (Maschinski & Haskins 2012). The actual success rate of plant reintroductions, however, is likely quite low (Godefroid et al. 2010). Long-term monitoring of the outcome of reintroduction efforts is infrequent, and the published literature reflects a strong bias toward positive results (Godefroid et al. 2010; Drayton & Primack 2012). In a meta-analysis, Godefroid et al. (2010) compared data from twenty-six published papers with results from a survey sent to 473 institutions that were suspected of having participated in reintroductions without publishing results. It was found that survival rates in the literature were much higher (78%) than those reported in survey data (33%), and that in studies where longer-term results were available, a startling decline in success occurred over time with an average of only 6% of reintroduced plants flowering after 4 years (Godefroid et al. 2010).

A variety of suspected and unknown factors affect the success of rare plant restoration efforts and the science itself is still young (Dalrymple et al. 2012). There is a great need for the development of techniques that will increase the viability of reintroduction efforts and research should occur at the species, ecotone, and global scales (Godefroid et al. 2010; Dalrymple et al. 2012). Greater understanding of the effectiveness and possible repurcussions of restoration methods at a variety of scales, and the variability within and between systems may allow practitioners to more easily and effectively implement successful projects, even where studies have not been conducted.

Restorationists working with rare and endangered species are often under pressure to produce short-term results due to the imminent threat of extinction. This can lead to haphazard restoration efforts that do not provide useful empirical data to guide future projects. Guerrant (2012) argues that regardless of outcome, reintroduction efforts need to be structured as designed scientific experiments in order to produce reliable and replicable results.

Long-term monitoring of reintroduction efforts and the publication of results is important as "failures" can often provide more-valuable information for the development of technical strategies than successes (Drayton & Primack 2012). In an unusual case where a reintroduction effort was recensused after 15 years, Drayton and Primack (2012) were surprised to discover that populations that were considered well-established three years after planting had almost entirely disappeared after 15 years. In the study, wildflower species planted in 1995 were resurveyed after two years and as seven of the eight species were present at reintroduction sites, leading the authors to publish the results as successful (Drayton & Primack 2000). In 2010, however, no surviving individuals of six of the eight species were found, and a seventh species was present at only one site, leading the researchers to conclude that long-term success rates for establishing new plant populations are very low, even when efforts are initially considered successful, and that research into factors that affect establishment over time is urgently needed (Drayton & Primack 2012).

Horticultural techniques that emphasize growth rates and short-term survival (one to two years) are often used in the cultivation of seedlings for restoration efforts. Seed stock, time, and funding are regularly in short supply, and propagating plants in

greenhouses with abundant nutrients, water, and lack of competition produces the largest number of seedlings for outplanting. However, high rates of failure occur when these propagules are introduced to stressful field conditions, which likely involve competition with other species, dense and low-nutrient soils, herbivory, drought, and pathogens (Godefroid et al. 2010; Haskins & Pence 2012). Restoration sites are by definition altered versions of the environments that plants evolved in, with additional anthropogenic stresses that often include invasive species, and changes to natural disturbance regimes, with soils affected by both these alterations and the restoration process itself.

Propagation methods that emulate field conditions can reduce the stress of acclimatization by providing plants with tools such as adaptive root structures and symbiotic partners that allow increased access to nutrients (Haskins & Pence, 2012). Nutrient stress may be especially problematic in restoration sites with a history of invasion. Non-native species often alter soil chemistry, for example, nitrogen-fixers have been shown to decrease soil phosphorus (Thorpe et al. 2013). Inoculation with mycorrhizal fungi often has positive benefits for plant survival, but the majority of research on mycorrhizal relationships has occurred in agriculture, horticulture, forestry, and basic science rather than conservation or restoration (Haskins & Pence 2012). The use of mycorrhizal fungi in the propagation of rare plants shows promise and may be especially suited to solving problems with acclimatization and long-term survival in reintroduction efforts (Gemma et al. 2002; Rowe 2007; Zubek 2008; Ferrazzano & Williamson 2013).

MYCORRHIZAL FUNGI

History and biology

Mycorrhizal fungi form symbiotic relationships with 80–90% of land plants and exist in nearly every terrestrial ecosystem (Smith & Read 2008). It is not through roots, but extensive mycorrhizal hyphal networks that plants uptake the majority of necessary nutrients, with autotrophs donating carbon to fungi in exchange for resources (Smith & Read 2008). Paleobotanical, morphological, and DNA-based evidence indicate that the earliest plants formed relationships with arbuscular mycorrhizal–like endophytes 400 million years ago, long before the evolution of roots (Brundrett 2002). Fungi likely originated over one billion years ago, predating terrestrial colonization (Smith & Read 2008). This ancient symbiosis heavily influences nutrient cycling, plant community structure, diversity, and soil characteristics today, and is considered to have a keystone ecological function globally (van der Heijden 1998; Jeffries et al. 2003).

Smith and Read classify mycorrhizal fungi into seven functional groups based on structural characteristics and autotrophic associates. Among these groups, the arbuscular mycorrhizal fungi (AMF) are by far the most abundant, and were recently organized into a separate fungal phylum, Glomeromycota, based on DNA sequencing (Schüβler et al. 2001). Unlike other mycorrhizal fungi, which are specific in host selection and only able to form partnerships with certain types or families of plants, AMF are generalists and capable of forming biotrophic relationships with an extremely wide range of autotrophs (Smith & Read 2008). AMF occur in almost all vegetated terrestrial areas and are the dominant mycorrhizal type in grasslands, tropical forests, and agricultural systems (Smith & Read 2008).

Morphologically, AMF consist of mycelium formed by masses of branching, threadlike hyphae that extend both into soil and within the roots of plants (Smith & Read 2008). Unlike ectomycorrhizal fungi, which form sheaths around root tips, AMF extend hyphae into and between the cortical cells of roots, forming intracellular arbuscules (branching tree-shaped organs) through which resources are passed bidirectionaly (Fig. 1; Smith & Read 2008).

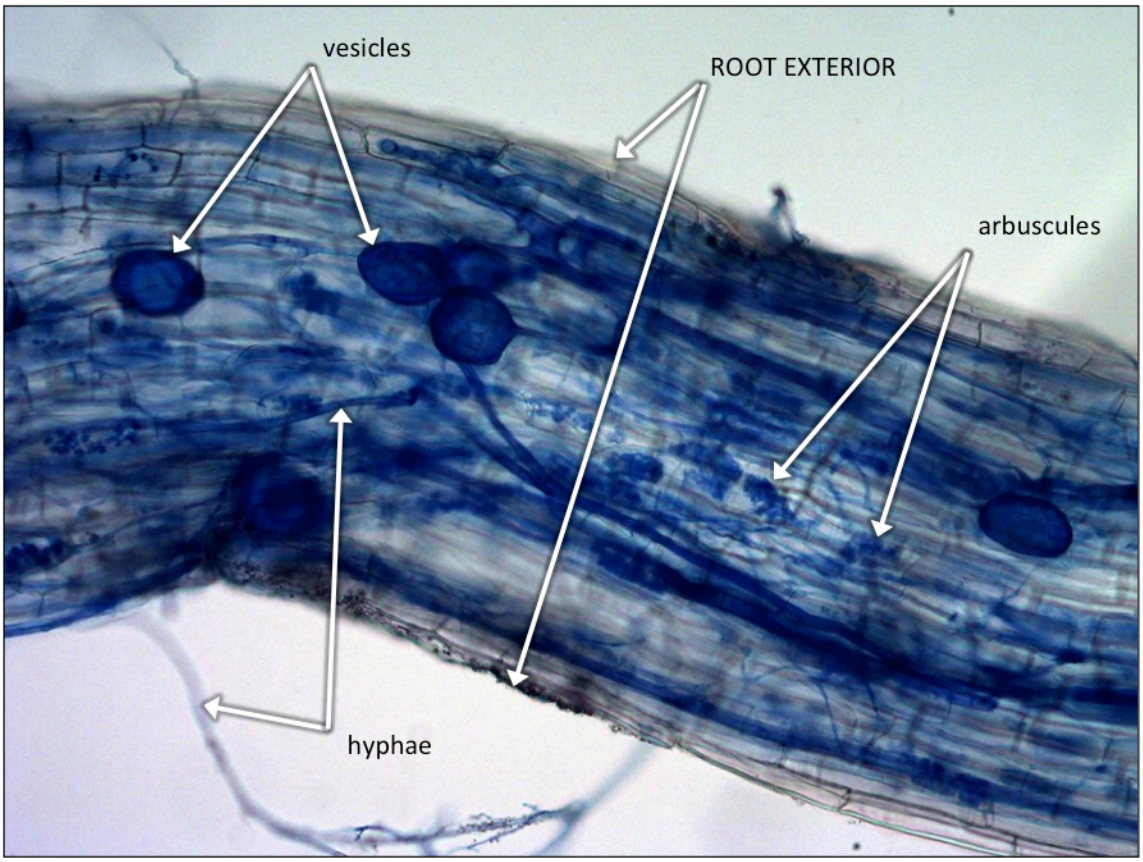

Figure 1. Microscopic view of a maize root (cleared) colonized by arbuscular mycorrhizal fungi (dyed blue). Structures within the root including vesicles, arbuscules, and hyphae, extraradical hyphae are also visible. Image adapted from Hazel Davidson, University of Aberdeen.

Unlike some saprotrophic fungi and ectomycorrhizae, under most circumstances AMF mycelia cannot be seen with the naked eye and do not form epigeous sporocarps of fruiting bodies such as mushrooms, but reproduce instead through large thick-walled spores spread by hyphae and fauna (Smith & Read 2008). Despite being very small (2–20 μm) AMF hyphae are extensive and ubiquitous, contain recalcitrant compounds, form unique conglomerates, live only 5–7 days on average, and thus likely contribute large quantities of organic carbon to soils (Staddon 2003; Smith & Smith 2011).

Researchers have only begun to tease out the actual genetic, cellular, and molecular interactions that allow the formation of mycorrhizae in the last decade or two, and details on what has been found could fill many much longer literature reviews (Smith & Read 2008). However, it is known that both AMF and plants respond to each other through a variety of complex signaling pathways and gene expression, which act in a coordinated manner to form mycorrhizae (Smith & Read 2008). Preformation signaling occurs, but precise mechanisms are not yet entirely understood, and this signaling is also stimulated (or potentially suppressed), by other microorganisms, such as the mycorrhization helper bacterium AcH 505 (Kurth et al. 2013). Interestingly, parasitic plants may have evolved to outilize the same pathways that mediate AMF recognition and colonization in host plants in order to exploit neighboring autotrophs as a carbon source without reciprocation (Fernández-Aparicio et al. 2010).

Mycorrhizal fungi are heterotrophic and rely on organic carbon from their photosynthetic partners (Smith & Read 2008). The entirety of resources that plants receive from this symbiosis, in contrast, is complex, and to what degree it is beneficial at any given time is not yet fully understood (Smith & Smith 2011). Water, phosphorus (P),

nitrogen (N), and trace minerals including copper (Cu) and zinc (Zn) are also involved (Smith & Read 2008). Isotopes have been used effectively in elucidating some of the details of nutrient transfer, but larger effects on plants such as growth, survival, diversity, hormone levels, architecture, tolerance to toxins, and disease- and drought-resistance also occur, but are less fully understood (Hartnett & Wilson 2002; van der Heijdan 2004; Smith & Smith 2012).

Reductionism has its place in understanding the benefits of mycorrhizal association to plants, but complexity theory and its tenets, including nonlinearity, positive and negative feedbacks, network connections, and emergent traits are extremely useful in understanding these relationships and their context within a larger ecological framework. The details of the transfer of benefits between symbiotic partners is an inherently complex relationship in a system that involves a variety of interacting dynamics. These are known to include plant variety and condition, the likely presence of many differently acting mycorrhizal species, changing resource levels, plant age and community, ecological conditions, the presence of other microorganisms and pathogens, and feedbacks between these and other unknown elements (Smith & Smith 2012; Hartnett & Wilson, 2002).

Plant interaction with AMF

In a systematic meta-analysis of plant response to mycorrhizal inoculation from 1,994 studies in 183 publications, Hoeksema et al. (2010) found a large variation in results with certain patterns emerging (Fig. 2). Plant functional group was the most important explanatory variable in AMF experiments, with (non-N-fixing) forbs exhibiting the

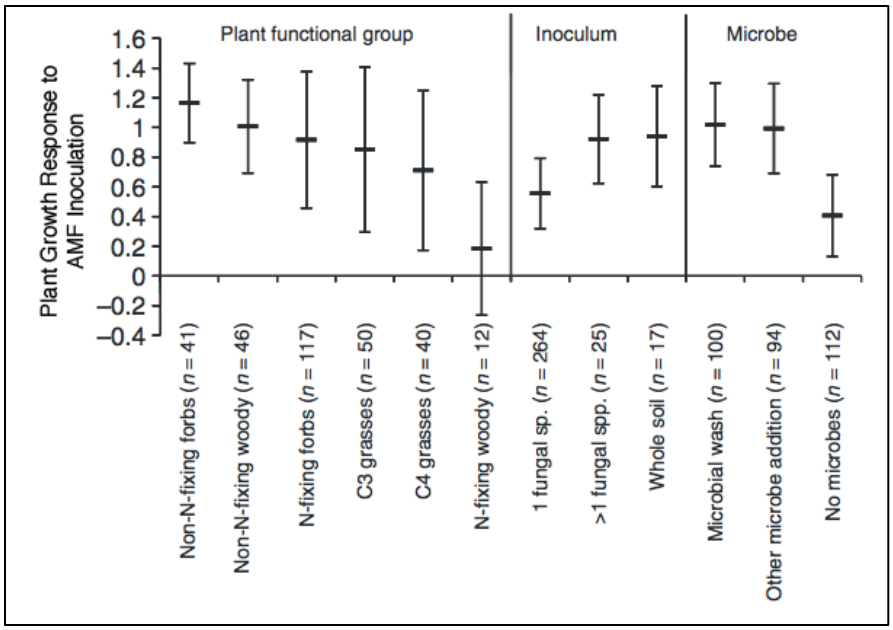

Figure 2. Trends from a meta-analysis show effects of other variables on plant response to arbuscular mycorrhizal inoculation. Figure from Hoeksema et al. (2010).

highest level of positive response to mycorrhizal association (Hoeksema et al. 2010). Whether plants were inoculated with one or more species of AMF was also important with inoculants containing more species promoting the greatest plant response, defined in this study as "the log response ratio of inoculated to non-inoculated plant biomass" (Hoeksema et al. 2010). The presence of other rhizosphere microorganisms significantly positively affected plant growth with AMF compared to sterile soil and shows that important and complex interactions occur between AMF, plants, and other members of the microbial community (Hoeksema 2010; Philippot 2013). Kurth et al. (2013) showed that a bacteria, AcH 505, is fungus-specific and produces growth regulators that significantly stimulate mycorrhizal formation when roots are inoculated with the microbe, which also leads to an increase in plant growth. Further, mycorrhization helper bacterium

have exhibited both a longer lifespan and increased abundance with greater MF presence (Kurth et al. 2013).

Hoeksema et al. (2010) found that N fertilization of soil significantly influenced plant response to AMF with growth showing a more positive response to fungal inoculation without fertilization with N. Where N is abundant, mycorrhizal relationships are reduced, likely because the symbiosis is less necessary for N acquisition. The introduction of N into ecosystems through either nitrogen-fixing invasive plants or agriculture can lead to a reduction in mycorrhizal fungi throughout the system (Vogelsang & Bever 2009, Thorpe et al. 2013). The majority of research on suppression of mycorrhizal fungi by N addition has occurred in agriculture, where the addition of N-based fertilizers suppresses AMF and changes systems from fungally to bacterially dominated, and requires the addition of greater amounts of P as non-mycorrhizal plants are less able to access this nutrient (Six et al. 2006). These changes in turn negatively affect soil aggregation, C storage, and nutrient leaching; these effects may increasingly spread from agricultural to wild systems with global changes to N cycling (Six et al. 2006; van der Heijden 2010; Asghari & Cavagnara 2012). AMF suppression through N fertilization is likely also problematic for plants grown in pots and then outplanted into environments that may have increased N from invasive plants, an agricultural history, or proximity to agriculture.

Plants show a high level of variability in response to AMF based not just on functional type, but individual plant species. AMF species can also affect response (Smith & Read 2008). Klironomos (2003) tested 64 plant species with an inoculant of one AMF species, *Glomus entunicatum,* and found plant growth responses that varied

from highly positive to highly negative (Figure 3). While the majority of responses were not significantly different from a non-inoculated control, a clear pattern of variability emerged when comparing among species. A negative growth response to AMF is always a possibility, and can occur when plants donate carbon for non-limiting nutrients.

Wilson and Hartnett (1998) found similar variability testing 95 tallgrass-prairie species in a greenhouse study. When categorized by plant functional type, forbs and C_4

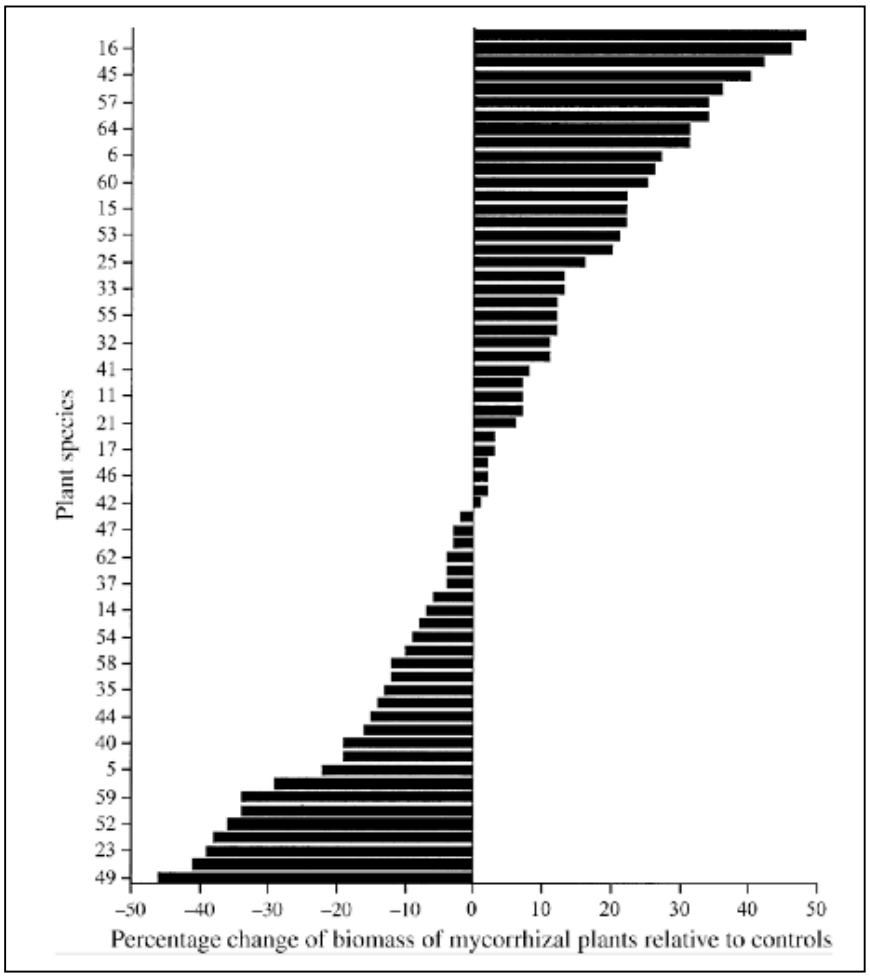

Figure 3. Sixty-four plants were inoculated with the arbuscular mycorrhizal fungi *Glomus etunicatum* and showed high variability in growth response compared to non-inoculated plants. While results from individual plants were not necessarily significant, the data show a clear trend of variability. Figure from Klironomos (2003)

grasses benefited most from AMF association, while C_3 grasses tended to have a neutral response and legumes (N-fixers) had a significant negative response (Wilson & Hartnett 1998). The high level of mycorrhizal obligation found in C_4, as opposed to C_3, grasses may be related to coarser root structures with a resultant decrease in nutrient acquisition ability, most prolific growth during summer, rather than spring, when water may be a limiting factor, and an abundance of C to allocate due to the more efficient photosynthetic pathway utilized by these plants.

Interestingly, native tall-grass prairie perennials were significantly positively affected by AMF while annuals showed lower root colonization and were not positively affected (Wilson & Hartnett 1998). The authors theorize that this is because annuals tend to thrive in newly or regularly disturbed areas where there may be less competition, and an advantage to growing very quickly without the need for long-term survival strategies. There also tends to be less AMF presence immediately after some disturbances, so annuals may have developed evolutionary strategies that were successful without AMF present, and that in fact gave a short-term advantage over late-successional species (Jasper 1991).

Response to AMF is complicated not only by plant type, but by interactions between plants and AMF species. In another experiment, Klironomos (2003) grew 10 plants with each of 10 AMF species and found that for a single plant response could vary from highly positive to highly negative depending on the mycorrhizal fungi species it was inoculated with. *Plantago lanceolata,* for example, showed a negative response of ~45% when inoculated with *Acaulospora morrowiae* and a positive response of ~45% when inoculated with *Glomus mosseae* (Klironomos 2003). No plant or AMF species

consistently responded either positively or negatively, and in fact all 10 species showed both positive and negative reactions to different AMF species, though not all of these results were statistically significant (Klironomos 2003). Interestingly, even genetically different individuals of the same AMF species affected plants in unpredictable ways and could cause a positive or negative response. Responses were most extreme with AMF genets from the site the seeds were collected from, as opposed to a foreign site (Klironomos 2003). The author concludes that variability in plant response to different AMF species may functionally maintain plant diversity on sites with multiple AMF species, but the exact mechanisms causing the variability are not well understood (Klironomos 2003).

Genetic variation within plant species can also affect response to AMF (Anderson & Roberts 1993). In a study of the prairie grass *Schizachyrium scoparium*, seeds from three locations were inoculated with AMF cultured from one of the source areas. Seedlings from the AMF-origin site and a nearby site (both in Mason County, IL) had significantly greater biomass than seedlings from a Nebraska source, and all three were larger than controls grown in sterilized soil (Anderson & Roberts 1993).

A theoretical model in which plant-mycorrhizal relationships are viewed as existing on a continuum between parasitism and mutualism has become widespread in the literature (Johnson et al. 1997). This model is useful in understanding variation in plant responses to mycorrhizal fungi, and rare cases where mycorrhizae formation causes clear negative effects, but the authors acknowledge that defining costs and benefits, especially at ecologically meaningful scales is difficult if not impossible (Johnson et al. 1997). The continuum approach is likely a simplification of a nonlinear relationship and not useful in

describing and predicting systems with high levels of complexity. The response of an individual plant to AMF can change throughout its lifetime, fluctuating from a positive to a neutral or negative response based on age, identity of the AMF, presence of pathogens or herbivory, and stressors such as drought (van der Heijden 2004; Smith & Read 2008). It is also a great simplification to define responses as positive or negative based only on the commonly used factor of growth rate, or economic models of nutrient exchange, when other morphological, qualitative, and unknown factors are also affected by mycorrhizal fungi (Smith & Read 2008; Hartnett & Wilson 2002). Smith and Smith (2012) also argue that even when there is a negative growth response, AMF are never truly parasitic because there appears to always be P transfer from AMF to plant, while parasitism implies unidirectionality of resources.

AMF and other microorganisms

Within a few centimeters of the roots of plants exists one of the most diverse and dynamic systems on Earth (Philippot 2013). In this highly complex interface, known as the rhizosphere, plants, fungi, bacteria, and other organisms interact with one another with effects that regulate the growth, composition, and biomass of plants, which directly or indirectly affect other organisms, making this one of the most important systems on the planet. Almost all organic nitrogen is first fixed by bacteria and archaea in the rhizoshpere, and carbon delivered by photosynthesizers enters this zone through roots and AMF (Chapin 2011). The intensified biogeochemical cycling of the rhizosphere combined with the multitude and variability of species and relationships that may be present make it a difficult area to study.

When conducting mycorrhizal research scientists often sterilize some portion of soil or growing medium to establish non-mycorrhizal control(s) (Koide & Li 1989). This methodology, however, also eliminates non-mycorrhizal organisms from control groups leading to unbalanced experiments if one treatment contains a whole soil community while another contains either no microorganisms or only added AMF without other microorganisms that would occur in natural soil. This problem can be exascerbated when whole-soil is sterilized, as elements, especially manganese, which can be toxic at high levels, are released by the autoclaving process (Koide & Li 1989).

Scientists have dealt with this issue in a variety of ways; one common method is to sterilize potting soil, and then add mycorrhizal inoculant to treatment groups, and a "soil microbial wash" to all groups. This wash is created by making a soil slurry with water, and filtering it through a sieve that is 38µm or less, which removes AMF spores but allows many other microorganisms to pass through (Koide & Li 1989). The resulting wash may contain beneficial microorganisms, such as mycorrhizal helper bacterium or pathogenic fungi such as rusts (Kurth et al. 2013). Microbial washes tend to have a beneficial effect on plant growth when combined with AMF (Hoeksema et al. 2010).

Ecosystem effects

AMF also have important effects at the ecosystem-scale (Rillig 2004; van der Heijden 1998; Wagg et al. 2011). In a study where 11 plant species were combined to simulate European calcareous grasslands, it was found that AMF species diversity significantly positively influenced plant diversity and ecosystem functioning (van der Heijden et al. 1998). Microcosms were inoculated with one of four native AMF species, a combination

of the four, or a nonmycorrhizal control, and it was found that low AMF diversity led to a few species becoming dominant at the expense of others (van der Heijden et al. 1998). A parallel macrocosm study simulating North American old-field ecosystems found that both plant biodiversity and ecosystem richness increased with the number of AMF inoculant species (van der Heijden et al. 1998). These combined results led the researchers to suggest that AMF should be considered as determinants of plant diversity in natural ecosystems (van der Heijden et al. 1998).

Hartnett and Wilson (1999) also found a strong effect of AMF on plant community diversity in a five-year tallgrass-prairie field study; however, this ecosystem experienced a significant decrease in plant biodiversity with mycorrhizae. Suppression of AMF by fungicide in the field resulted in a large increase in plant species biodiversity, and no change in aboveground biomass (Hartnett & Wilson 1999). The authors theorize that the elimination of AMF led to a decrease in obligately mycotrophic C_4 tall grasses, with subsequent increases in subordinate C_3 grasses and forbs (Hartnett & Wilson 1999). A secondary finding indicates that across the five-year study period, annual precipitation was negatively associated with mycorrhizae in that AMF root colonization increased with decreasing annual precipitation. While the mechanism behind an increase in colonization was likely because plants were better able to access water, a limiting resource, through more extensive AMF networks, this also further elucidates the complexity of mycorrhizal symbiosis (Hartnett & Wilson 1999).

More recently, Vogelsang et al. (2006) found that plant diversity and productivity were more responsive to AMF identity rather than diversity. In addition, the authors found that complex interactions occur among AMF species and P sources that alter

community-level ecosystem properties. Increasing types of P source (from one to five, both organic and inorganic) added to the ability of AMF to promote diversity in plants (Vogelsang et al. 2006). AMF presence and diversity was found to reduce plant–plant competition in a greenhouse co-planting study (Wagg et al. 2011). Four AMF species were tested individually and in combination to elucidate growth dynamics between two plants. AMF diversity was shown to reduce competition by reducing the growth suppression effects of a grass on a legume in soils of varying quality, and the authors suggest that a species-rich AMF community may act as insurance in maintaining plant productivity in a fluctuating environment (Wagg et al. 2011).

The body of work on the ecosystem effects of mycorrhizal fungi is young, evolving, and undergoing a rapid increase, and sweeping conclusions cannot be drawn, yet it is clear that AMF play a significant role in shaping and maintaining plant communities. There appear to be complex feedbacks regulating these relationships, and it has also been shown that plant community composition can affect AMF community diversity (Eom 2000; Hausmann & Hawkes 2009). Greater AMF diversity seems to create emergent and self-organizational effects, regulating ecosystems in a wide variety of ways, as well as increasing growth effects on individual plants (van der Heijden et al. 1998; Hartnett & Wilson 1999; Wagg 2011; Hoeksema 2010).

COMMON MYCORRHIZAL NETWORKS

A single AMF may colonize more than one plant, creating a common mycorrhizal network (CMN) and greatly increasing the complexity of symbiotic dynamics (Selosse 2006). Research indicates that plants share nutrients, water, and signals through CMNs

(Egerton-Warburton et al. 2007; Song et al. 2010). In an experiment with tomato plants linked only by a common mycorrhizal network, Song et al. (2010) found that when one plant was exposed to a pathogen the neighboring plant, which had contact only through mycorrhizae, released at least six enzymes and induced six genes related to defense. Collectively plants are known to produce a staggering variety of root exudates, perhaps in excess of 100,000 (Bais et al. 2004). Research by Song et al. (2010) and others shows that plants are able to exchange these signals through CMN, likely incurring useful warnings and benefits in a form of "communication" unavailable to plants that are not part of CMNs (Simard et al. 2012). This sharing of signals may be one reason plants associate with AMF even when it does not appear to be beneficial based on growth rate or nutrient economics.

Much of the initial evidence and proof of the existence of CMNs comes from studies of mycoheterotrophic (MH) plants (Simard et al. 2012; Courty et al. 2011). MH plants live in the forest understory and obtain C through the exploitation of CMNs maintained by photosynthesizers; this C-obtaining strategy has evolved independently several times and some plants are also known to be partially MH, heavily supplementing photosynthesis with resources from a CMN (Courty et al. 2011). In a recent study of MH plants in the tropics, Courty et al. (2011) analyzed the stable isotopes ^{15}N and ^{13}C to examine the nutritional dynamics of MNs. AMF have been shown to be depleted in the stable isotope ^{13}C by ~ -2‰ to -4‰ compared to their host plants, and MH plants reflect the $\delta^{13}C$ signatures of CMN rather than nearby plants (Etcheverria et al. 2009; Courty et al. 2011; Walder et al. 2013). This change in ^{13}C indicates that carbon is being transferred from the host plant to a MN and subsequently to the MH plant. These isotope studies

effectively show that plants are able to indirectly obtain resources from other plants via CMN, and results from lab and field studies using autotrophs linked by CMN have shown bidirectional resource transfer (Simard et al. 1997, 2012; Philip et al. 2010).

Nutrient transfer

In a series of experiments in the late 1990s a British research group analyzed C transfer between autotrophs linked by a CMN through the analysis of $\delta^{13}C$ signatures (Fitter et al. 1998; Watkins et al. 1996; Graves et al. 1997). Plants that use a C_4 photosynthetic pathway have distinctly more enriched $\delta^{13}C$ signatures (*Cynodon dactylon* ~ -14‰) than C_3 plants (*Plantago lanceolata* ≈ -28‰) and this difference has been effectively used to trace the C transferred through CMNs (Sage & Monson 1999). C_4 photosynthesis has emerged several times evolutionarily in different parts of the world, is considered more efficient than C_3 photosynthesis, and occurs in the majority of grasses, as well as crops such as maize, sugarcane, millet, and sorghum (Sage & Monson 1999). Co-planting experiments using C_3 and C_4 plants linked by a CMN took advantage of the natural difference in $\delta^{13}C$ signatures to effectively show that autotrophs transfer C to other plants through CMNs (Fitter et al. 1998; Watkins et al. 1996; Graves et al. 1997). Whether or not the majority of C transferred to plants via CMN stays in roots or enters shoots is still debated among scientists, and it is clear that at different life stages plants may benefit from C exchange more than at others (Simard et al. 1997, 2012; Philip et al. 2010).

A recent greenhouse experiment by Walder et al. (2012) elegantly showed that plants do not contribute to and receive benefit from CMNs equally. A C_3 plant, *Linum usitatissimum,* and a C_4 plant, *Sorghum bicolor,* were coplanted with mycorrhizae and in

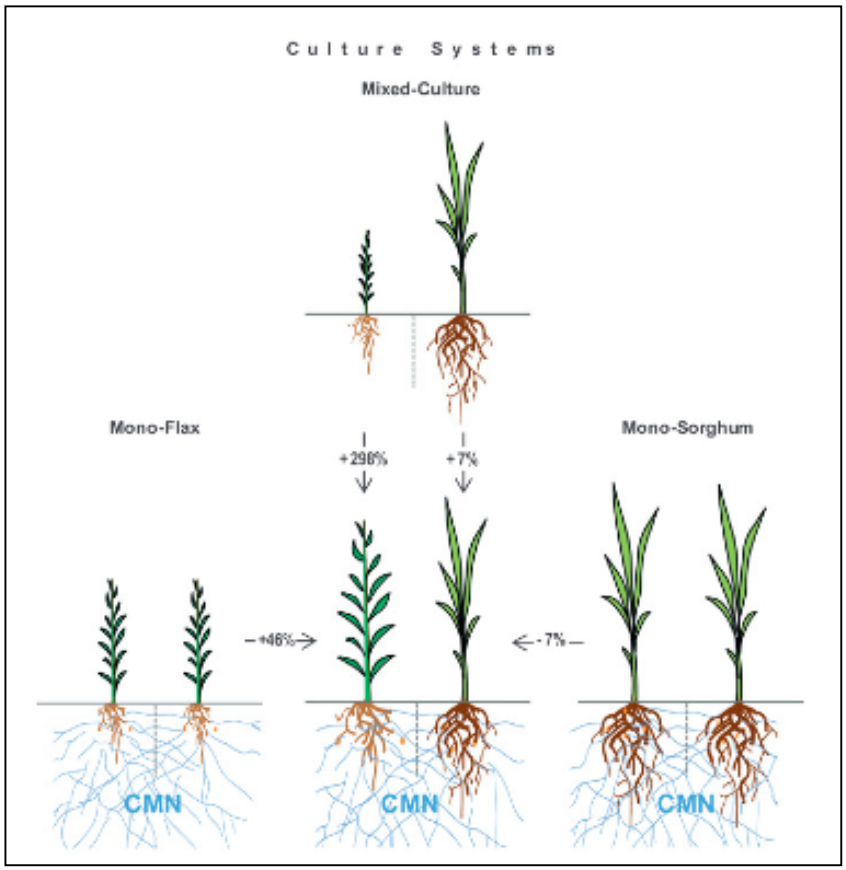

Figure 3. Results of inoculation with a common mycorrhizal network (CMN) on growth of a C_3 plant, flax (*Linum usitatissimum*), and a C_4 plant, sorghum (*Sorghum bicolor*). Flax benefits positively from both the CMN, and the relationship with sorghum, while sorghum experiences only small changes in growth (Walder 2012).

monoculture with and without AMF (Walder et al. 2012). Hyphae from the CMN were exposed to the stable isotopes ^{15}N and ^{33}P as tracers in a separate hyphal compartment divided from the roots by a 25µm mesh screen that AMF hyphae, but not plant roots, could cross (Walder et al. 2012). It was found that with inoculation of *Glomus intraradices* the C_4 plant donated 90% of the C to the CMN and received only 10% of the N and P exchanged (Walder et al. 2012). Inversely, the C_3 plant donated only 10% of the total C to the CMN yet received 90% of the N and P from the network (Fig. 3; Walder et al. 2012). This unequal relationship resulted in a 298% positive change in growth for *L.*

usitatissimum, the C3 plant (Walder et al. 2012). A similar pattern, though less extreme in disparity occurred with inoculation of a different AMF species, *Glomus mosseae* (Walder et al. 2012). These results show clearly that autotrophs can exploit CMNs through unequal contribution to and benefit from networks, regardless of whether C is actually significantly assimilated into shoot matter, and contradicts assumptions that "fair trade" exist in CMN symbiosis. Walder et al. (2012) theorize that C4 plants have a carbon excess, and thus are trading in "luxury goods," which are not actually needed, but that these results may differ over the whole lifecycle of plants. The C_4 species involved in the experiment actually lost very little in terms of biomass compared to the monoculture, but greatly improved the growth of the C_3 plant, showing again that relationships are nonlinear, involve feedbacks, and lead to facilitation and emergent qualities, such as the 298% increase in growth of the C_3 plant with only a 7% reduction in the growth of the C_4 species (Walder et al. 2012).

While it is still unclear how CMNs affect plants in the field, these networks may be key to some relationships with bidirectional transfer affecting species composition. In Canada, it was found that Douglas fir (*pseudotsuga menziesii*) and paper birch (*Betula papyrifera*) exchange carbon through ectomycorrhizal fungi in a season-specific pattern that benefits both species (Philip 2010). When *P. menziesii* was shaded in the summer, *B. papyrifera* transferred more carbon to the conifer, and *P. menziesii* reciprocated by donating C to the deciduous tree in fall and spring (Philip 2010). Bidirectional transfer of resources may help to maintain diversity and stability in ecosystems, and re-establishing these relationships through the reintroduction of mycorrhizal fungi could be key to successful restoration efforts.

AMF AND RARE PLANT REINTRODUCTION

Smith and Read (2008) argue that mycorrhizal colonization is normal for plants, and that existing in a non-mycorrhizal state (NM) should be considered abnormal. The NM condition occurs only under special circumstances, such as when a plant is one of approximately 10% of species that are considered NM, where extreme disturbance to soil has occurred, for example in sites degraded by mining, or when plants are grown in pots and not inoculated with mycorrhizae (Smith and Read 2008). Therefore, plants grown in greenhouses for reintroduction without mycorrhizae should generally be considered to be developing under abnormal conditions and likely have differences in architecture, growth rate, and pathogen resistance compared to mycorrhizal counterparts (Haskins & Pence 2012).

Several researchers have investigated using AMF inoculant in the propagation of native, rare, and endangered plants in the greenhouse for eventual reintroduction, with successful results (Haskins & Pence 2012). In Arizona, Richter and Stutz (2002) inoculated *Sporobolus wrightii,* a formerly dominant grass species in semi-arid riparian floodplains, with local AMF in a greenhouse experiment. Previously *S. wrightii* had been directly seeded with virtually no success, and only mixed success had been seen for propagation and transplanting. Seedling emergence in the greenhouse was higher in inoculated pots, and more tillers were produced in inoculated plants grown in small pots, though growth was not affected (Richter & Stutz 2002). After transplant to the field, *S. wrightii* seedlings propagated with AMF showed greater survival, basal diameter, and tiller and panicle production through the first two growing seasons, which was the duration of subsequent monitoring (Richter & Stutz 2002). These results highlight the

potential of AMF inoculation for restoration, the importance of focusing on factors other than growth in determining success, and the need for long-term monitoring of reintroduced plants. Interestingly this study also found that plants started in smaller containers had greater survival, height, basal diameter, and tiller production (Richter & Stutz 2002).

Hawaii is home to 41% of endangered plant species in the US (as of 2002) and propagation in greenhouses and later reintroduction of seedlings to the field has been an important and mostly unsuccessful element of conservation (Gemma et al. 2002). Four endemic species, two of which are listed endangered, were grown with local AMF inoculant, and growth of both roots and shoots was significantly enhanced in all species tested compared to control plants in the greenhouse (Gemma et al. 2002). Growth was especially enhanced in low-P soils, and P limitation is widespread in Hawaii (Gemma et al. 2002). No outplanting or long-term survival data were included in this paper; however, the authors suggest that the strong positive plant response to inoculation may indicate greater potential for field success.

In an AMF inoculation trial of six native montane species from Rocky Mountain National Park (CO), Rowe et al. (2007) found a significant though varying growth response to inoculation. Three late-successional species showed a positive response to AMF, while three early successional species showed a negative response (Rowe et al. 2007). Both native and commercial AMF inoculants were tested, and native-cultured inoculant produced higher levels of root colonization and plant response (Rowe et al. 2007). Soil P levels had an effect on AMF responsiveness for only one plant studied (Rowe et al. 2007).

Three plant species, two of which are on the International Union for Conservation of Nature (IUCN) Red List, meaning that they are of highest conservation priority, and one of which is extinct in the wild in Poland, were tested for response to native AMF, a mixture of laboratory AMF strains, and a combination of laboratory strains and rhizobacteria in Europe (Zubek et al. 2009). Inoculation type did not have a significant effect, but all three plants were shown to be dependent on AMF with two having extreme 19- to 22-fold and 11- to 14-fold gains in biomass over non-mycorrhizal controls (Zubek et al. 2009). The authors conclude that AMF inoculation should be used in the propagation of these species and that it is likely to aid in future success of outplanting and reintroduction (Zubek et al. 2009).

In climate-change-related reintroduction research, Ferrazzano and Williamson (2013) inoculated seeds of an endangered plant with AMF and planted the *Abronia macrocarpa* seeds directly into plots in an area of Texas that was experiencing drought (2013). Growth factors, including mean number of leaves and mean aerial diameter were significantly greater in AMF-treated plants (Ferrazzano & Williamson 2013). This study shows not only that AMF inoculation can aid *A. macrocarpa,* but that mycorrhizal colonization of plants may become increasingly important under global warming.

The results of these five studies all show positive response of rare plants to AMF inoculation for at least some of the species studied, and indicate that AMF inoculation is a strategy that should be tried on more species, in different ecosystems, and with long-term survival monitoring. It is important to keep in mind, however, that a positive-results bias exists in data reporting and publishing, and that these data are likely not indicative of the total research that has occurred in studies of mycorrhizal inoculation and rare plant

reintroduction (Heidorn 2008). This important research should continue, and both positive and negative results should be shared publicly. Widespread sharing of all results is becoming more possible as organizations increasingly host websites where reintroduction success, failure, and methods data may be submitted anonymously and accessed through databases (Guerrant 2012). In addition, land managers who decide to test and use mycorrhizal inoculation should be aware of the complexities of both mycorrhizal symbiosis and plant reintroduction and the need to collect and consider data beyond initial growth rates, and that not all species will respond in the same way.

Inoculant source

It has been shown that different species of AMF affect plants differently, and that these effects occur not only based on species, but genotype (Anderson & Roberts 1993; Klironomos 2003). Whole soil containing inoculant collected from local sites is a potential source of AMF that has been well-studied. Rowe et al. (2007) found mycorrhizal colonization of 100% of plants treated with field soil and only 8.4% of those that received a commercially available inoculant. Unfortunately local whole-soil inoculants, while cost-effective, are not always available and can include unwanted pathogens. In ecological restoration it can be especially problematic to remove soil from intact sites, which could cause damage, especially if done regularly or in large amounts. Degraded sites may contain few AMF or an altered species composition (Vogelsang & Bever 2009). In some cases, soil from high-quality sites may be almost completely unavailable, such as in the prairies of South Puget Sound, Washington, where the highest

quality remnant patches exist on a military base and digging is not allowed due to the presence of unexploded ordinances.

Samples of AMF can be collected from the roots of native plants, isolated, and added to sterilized media in "pot cultures" (IJdo et al. 2011). This method reduces the need for field soil, and the possibility of unintentionally introducing pathogenic organisms. It can, however, take years to produce sufficient inoculant for large-scale use, and cultures have a tendency to become reduced in AMF species complexity over time. The phenomenon is due to unintentional selection for AMF that thrive in a greenhouse environment and are able to competitively colonize the plant species grown in cultures (IJdo et al. 2011). Accidental contamination with non-native AMF species can also occur, and is difficult to detect.

Commercial inoculants are a readily available and appealing source of AMF, and contain species that are known to grow quickly and colonize a wide variety of plant species. These "general" inoculants also have disadvantages. The quality is variable, and difficult to test, and species that have the best initial colonization abilities in the greenhouse may not be the most beneficial for long-term growth in the field (Schwartz et al. 2006; Rowe 2007). Introducing non-native AMF species into the field is also a very real, though not well-studied possibility (Schwartz et al. 2006). Plants inoculated with AMF in the greenhouse tend to retain that AMF community identity, even after planting in field soil (Mummey et al. 2009).

Risk

Human-enhanced movement of plant, animal, and pathogen species around the world through globalization has had devastating ecological, social, and economic consequences. Many habitats have become severely degraded, leading to poor functioning and reduced ecological services, and invasive species have been a major force, along with habitat loss, in causing extinctions (Schwartz et al. 2006). While no known problems from the introduction of non-native AMF have been documented, Schwartz et al. (2006) note that this may be due to size. Human ability to notice and record invasions tends to correlate with the size and therefore visibility of the invasive organism, vertebrates tend to be quickly noticed, while very little is known about the history of earth worm invasions, despite their effects on ecosystems in North America. Exceptions to this rule of size do occur, fore example when larger species that we consider important are visibly affected, such as with the diseases that caused the chestnut blight or potato famine. AMF, however, are both invisible to the human eye and not well-documented to begin with, leading to the very real possibility of negative effects of invasion going unnoticed.

Introduction of non-native AMF could lead to both biological and chemical changes to ecosystems with potentially global consequences. Unlike AMF, ectomycorrhizal fungi have been known to become invasive and alter biogeochemical cycles. In Ecuador, many highlands that once contained paramo grasslands have been planted with non-native radiata pine (*Pinus radiata*) saplings that had been grown in soil containing EMF inoculum from older pines (Chapela et al. 2001). Conversion of grasslands to forest leads to an increase in carbon stored in aboveground biomass and has

received attention as a mitigation strategy to combat global warming, under the assumption that belowground C would not change. However, researchers found a loss of up to 30% of soil C twelve years after plantation (Chapela et al. 2001). Through a variety of techniques including stable isotope and EMF genetic analysis, Chapela et al. (2001) found that EMF diversity was extremely low compared to native radiata pine forests, and that the fungi were acting saprotrophically, effectively leading to "photosynthesis-derived soil C mining." Carbon from the soil C pool, established by grasslands and AMF, was being turned into an abundance of fungal fruiting bodies, with productivity of *Suillus luteus* alone up to three orders of magnitude greater than all EMF combined in comparable native forests (Chapela et al. 2001). This study shows the drastic and unexpected effects that non-native mycorrhizal fungi can have on an ecosystem and its biogeochemical cycling.

AMF, regardless of nativity, may also benefit invasive plants. While AMF densities have been shown to decline in association with invaders, and low densities contribute to invasion, many common weeds have been shown to respond positively to AMF (Vogelsang & Bever, 2009). AMF networks can also work against native plants, as was found in an experiment where spotted knapweed (*Centaurea maculosa*), a problematic invader, was shown to experience maximum growth benefit with a native fescue (*Festuca idahoensis*) and AMF, in comparison to trials without AMF or with another noxious weed, even though *C. maculosa*'s photosynthetic rate was 14% lower than when it was grown alone (Carey et al. 2004).

While AMF inoculation does carry risks, it is important to remember that no serious damaging effects have yet been documented, and that all management activities

carry risk, as does a lack of management. AMF, and especially native cultured AMF are likely much less risky than other practices such as pesticide use, and may well help to save species from extinction.

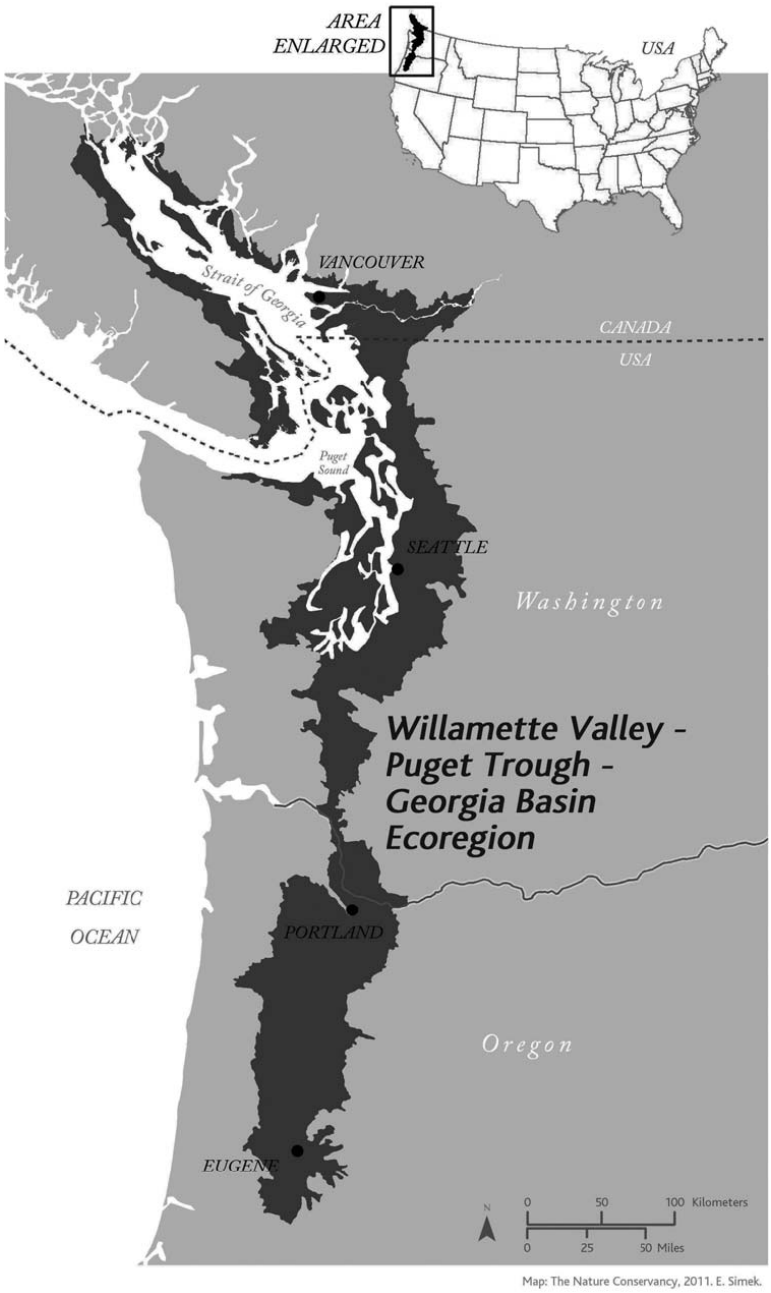

Figure 4. Geographical extent of the Willamette Valley–Puget Trough–Georgia Basin Ecoregion. Historically, much of this area was dominated by prairies and oak savannas maintained through anthropogenic use of fire. Map from Hamman et al. 2011.

PACIFIC NORTHWEST PRAIRIE-OAK ECOSYSTEMS

The prairie and oak savannas of the Willamette Valley–Puget Trough–Georgia Basin (WPG) ecoregion are among the most endangered ecosystems in the United States (Floberg 2004; Dunwiddie & Bakker 2011). This ecoregion is located in the Pacific Northwest, and occurs between the Cascade Range and coastal mountains from southern British Columbia to southern Oregon, USA (Fig. 5) (Hamman et al. 2011). While it is difficult to quantify the original extent of these ecosystems, most experts agree that <10% of these habitats remain, and that <5%–1% are considered "intact" and dominated by native species (Dunwiddie & Bakker, 2011; Hamman et al., 2011). A variety of factors have led to the disappearance and degradation of WPG prairies including non-indigenous settlement, woody species encroachment, the introduction of non-native plants, and most importantly, the extirpation of indigenous people and their land management practices (Boyd 1999).

Overwhelming evidence, including historical narratives, charcoal and pollen records, dendrochronology, plant physiology, ecosystem ecology, and indigenous knowledge, indicate that WPG prairies and oak savannas were shaped and maintained by the intentional use of fire (Cooper 1859; Boyd 1999; Hamman 2011; Sprenger et al. 2011; Walsh et al. 2010). Regular anthropogenic use of burning in the WPG is thought to have ceased in the mid-nineteenth century when lands were heavily settled by Euro-Americans and converted to agriculture. Both historical records and charcoal evidence suggest, however, that in many parts of the WPG region anthropogenic burning slowed or ceased around 1700, due to indigineous mortality through disease, and that the landscapes first viewed by settlers around 1850 were already heavily altered by 150 years of woody

encroachment and Native American–population decline (Cooper, 1860; Walsh et al., 2010). Restoration of the prairie and oak savannas of the WPG, and the species that evolved and coexisted under indigenous use of high-frequency, low-severity fire, is the preservation of not just a biological landscape, but a cultural one.

Several species that are endemic to WPG prairies have been listed or are candidates for threatened and endangered status, according to the ESA including: the hemiparisitic golden paintbrush (*Castilleja levisecta*), the Taylor's checkerspot butterfly (*Euphydryas editha taylori*), Mardon skipper (*Polites mardon*), streaked horned lark (*Eremophila alpestris strigata*), and Mazama pocket gopher (*Thomomys mazama*) (Dunwiddie & Bakker, 2011). More than 100 species associated with WPG prairie and oak savannas are considered "at risk" in British Columbia (Dunwiddie & Bakker 2011). Forty-six plant species that are fire-adapted or fire-dependent and specific to the WPG prairie and oak savannas are either critically imperiled, imperiled, or vulnerable (Hamman 2011). Protection of biodiversity has made these prairie and oak savannas a high priority in the region for both research and conservation efforts, which have been underway and steadily maturing in both scope and technique over the last twenty years (Dunwiddie & Bakker 2011).

Regional restoration efforts include the use of prescribed fire and species reintroductions, and appropriately sourced seeds and seedlings are produced in large quantities for reintroduction (Hamman 2011). Plant reintroduction efforts suffer from the same problems with long-term establishment that plague restoration projects globally (Hamman, personal communication). It appears that AMF have not been studied in the WPG prairie oak savanna ecoregion, with two exceptions. The first is a master's thesis by

Sierra Smith that focused on the reconversion of former agricultural land to prairie (2007). *Achillea millefolium* and *Festuca scabrella* were grown in soil with or without AMF in a greenhouse, and negative growth effects occurred, though interestingly a trend toward greater vigor in AMF seedlings was also found (Smith 2007). A second study in South Puget Sound, WA, tested inoculation of several plants, but issues with seed germination occurred and fertilizer was added to non-AMF control plants, making data comparison problematic. Nevertheless, initial field results indicate that AMF-inoculated plants are experiencing greater survival than their fertilized counterparts (Hamman, personal communication).

WPG prairie plant species are excellent candidates for mycorrhizal inoculation and long-term success, due to low native soil N and P, and other restoration efforts that are occurring, including regular use of prescribed fire (Hamman, 2011). A large number of groups including nonprofit conservation organizations, government agencies (federal, state, and local), and Joint Base Lewis-McChord (the landholdings of which include the largest remnants of intact prairie in the ecoregion), are heavily invested in WPG prairie-oak restoration (CNLM, 2013). A portion of seed produced is dedicated to research efforts, and the major financial and labor involvement of a wide-range of stakeholders makes long-term monitoring of experimental efforts possible (Sarah Hamman, personal communication, 2013). Research into the effects of AMF in the propagation of rare and native WPG prairie plants will potentially address issues of both basic and applied science at a variety of scales, and inform restoration and conservation efforts globally.

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Mycorrhizal and microbial inoculation affect the growth and survival of native plants raised for restoration

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Key words: arbuscular mycorrhizae, native plants, restoration, soil microbial community, Willamette Valley–Puget Trough–Georgia Basin prairie, greenhouse propagation

Running title: Mycorrhizal and Microbial Inoculation

Implications for practice:

- Use of arbuscular mycorrhizal fungi inoculants in the greenhouse can enhance seedling growth and survival
- An AMF inoculant cultured from the roots of local native plants worked as well as a commercially available one
- Care should be taken with whole-soil, rather than cultured inoculants, which may contain pathogens and decrease seedling emergence, survival, and growth

ABSTRACT

Production of native seedlings for field outplanting has become a common ecological restoration technique worldwide. However, the establishment of greenhouse-raised plants in the field is usually poor. Mycorrhizal fungi are symbionts that can provide survival benefits to host plants. This relationship is ubiquitous in terrestrial ecosystems and mycorrhizae are absent only under unusual circumstances, such as in a nursery greenhouse.

In this study, nine Northwest short-grass prairie species were grown for six months in sterilized medium with an arbuscular mycorrhizal fungi (AMF) inoculant cultured from local native plants, a general AMF inoculant, or in control treatments. Three microbial inoculants with AMF removed, created from a nearby site considered to be high-quality remnant prairie, a restoration site, and unsterilized potting medium, were added within each AMF treatment in a full factorial design. Seedling emergence, survival, aboveground growth, and biomass data were collected.

AMF significantly enhanced the growth of five species and the survival of four, with no detectable effect on the remainder. Further, there was no significant difference between the two AMF inoculants. Field microbial wash tended to have a negative effect on seedling emergence and growth, with the high-quality site treatment most repressive. AMF and the introduced microrganisms interacted on *Festuca roemeri,* with AMF mediating the negative effect of other fungi. Surprisingly, AMF positively affected the growth of *Castilleja levisecta,* a hemiparasite, and altered the phenology of *Dodecatheon hendersonii,* delaying dormancy. These results suggest that AMF can enhance the growth and survivorship of many species, and that inoculation may lead to greater success in ecosystem restoration efforts.

INTRODUCTION

Many scientists agree that we are in the midst of Earth's sixth great extinction event, characterized by the loss of important natural and cultural landscapes, and a steady decline in biodiversity globally (Barnosky et al. 2011). Encouraging the re-establishment of viable wild populations of rare and native plant species in degraded ecosystems through cultivation and outplanting is a widespread restoration technique. Plant reintroduction attempts to mitigate loss of biodiversity and to prevent extinction by increasing native plant abundance and diversity, with a resultant preservation of species

across trophic levels. The actual success rate of plant reintroductions, defined by Godefroid et al. (2011) as the ability of a population to persist and reproduce, however, is quite low; long-term monitoring of the outcome of reintroduction efforts is infrequent, and the authors found that published literature reflects a strong bias toward successful results (78%), as compared to those reported in survey data (33%). Where longer-term results were available, a startling decline in success occurred over time with an average of only 6% of reintroduced plants alive and flowering after 4 years (Godefroid et al. 2011).

The failure of greenhouse-raised transplants is due in part to horticultural techniques that provide abundant nutrients but poorly emulate native environments (Haskins & Pence 2012). Field soils contain complex microbial communities capable of altering plant growth and survival while growing media often lacks biota or contains communities that are compositionally distinct from those in outplanting sites. Lack of mycorrhizal fungi, symbiotic partners with which plants evolved, may be especially problematic for greenhouse-raised perennial seedlings (Haskins & Pence 2012).

Arbuscular mycorrhizal fungi (AMF) consist of mycelium formed by masses of branching, threadlike hyphae that enter roots and receive carbon from the host plant in exchange for extended access to resources; it is through AMF, not roots, that the majority of nutrients are taken up by plants (Smith & Read 2008). AMF have been shown to affect plant growth, survival, diversity, hormone levels, morphology, tolerance to toxins, herbivory, disease, and drought (Hartnett and Wilson 2002, van der Heijden 2004, Smith & Smith 2012). These effects are influenced by factors such as plant species, age, community composition, AMF species and diversity, resource levels, edaphic biota, and

feedbacks between these and other complex elements (Klironomos 2003, Hoeksema et al. 2010, Smith & Smith 2012). Some plants cannot survive without colonization, while others, especially annuals and early successional species, may show a negative growth response to AMF because plant carbon is traded for non-limiting nutrients (Smith & Smith 2012). Degraded environments often contain less AMF, so inoculation in the greenhouse could give transplants not only an equal playing field, but an advantage over neighbors (Vogelsang & Bever, 2009).

Previous greenhouse studies of AMF effects on the propagation of native, rare, and endangered plants have shown positive effects on four species in Hawaii (Gemma et al. 2002), two forbs in Florida (Fisher & Jayachandran 2002), a grass in Arizona (Richter & Stutz 2002), three late-successional species in subalpine Colorado (Rowe et al. 2007), and three montane species in Poland (Zubek et al. 2009). Seedlings were outplanted in only one of these studies; for the study period examined (two growing seasons) Richter and Stutz (2002) found that AMF inoculation led to increased survival, basal area, and tiller and panicle production. While the current literature indicates positive effects, implementation in conservation lags behind agriculture and horticulture, where use is more common (Haskins & Pence 2012).

For ecological and economic reasons conservationists may be especially concerned with the source of mycorrhizal inoculants, which can be produced from native ecosystem-specific AMF strains, sourced from generalized commercially available AMF communities, or introduced by adding small amounts of whole-soil to growing medium (Schwartz et al. 2006). While AMF have not been known to become invasive, Schwartz et al. (2006) recommend using inoculants cultured from local sources to avoid

introducing nonnative strains to field environments. Further, individual plant species have growth responses that vary from highly positive to negative based on AMF species identity and source, with local genotypes producing the strongest reactions (Klironomos 2003). In addition, the presence of other microorganisms, such as bacteria, tends to increase plant response to AMF through complex interactions including increased nutrient cycling and the presence of mycorrhization helper bacterium (Hoeksema et al. 2010, Kurth et al. 2013). We hypothesized that native cultured AMF combined with a microbial community from a high-quality prairie site would lead to greater plant growth and survival than a general AMF inoculant, or microbes from other sources.

Prairie and oak savannas of the Willamette Valley–Puget Trough–Georgia Basin (WPG) ecoregion in the Pacific Northwest are among the most endangered ecosystems in the United States with <10% remaining, and only between 1-5% dominated by native species (Dunwiddie and Bakker 2011, Hamman et al. 2011). A variety of factors have led to the disappearance and degradation of WPG prairies including increased settlement and agriculture, invasion by trees and non-native plants, and the extirpation of indigenous people and their land management practices, which included the regular application of fire. US Endangered Species Act (ESA)–listed organisms in the area include the hemiparisitic golden paintbrush (*Castilleja levisecta*), Taylor's checkerspot butterfly (*Euphydryas editha taylori*), streaked horned lark (*Eremophila alpestris strigata*), and Mazama pocket gopher (*Thomomys mazama*). Further, 46 plant species that are fire-adapted or -dependent and specific to the WPG are either critically imperiled, imperiled, or vulnerable (Hamman, 2011).

This study addressed the use of AMF inoculation for the production of restoration seedlings by testing nine native WPG species with two AMF inoculants and three microbial cultures. A secondary goal was to produce sufficient material for outplanting and long-term study. The following questions were addressed 1) How does AMF inoculation affect the growth of greenhouse-raised seedlings? 2) Does AMF affect short-term (6 months) survival? 3) Is an AMF inoculant cultured from native soils superior to a commercially available one? 4) How will AMF inoculants interact with different soil microbial communities likely to be present in outplanting sites?

MATERIALS AND METHODS

Seed stratification and sowing

Seeds of *Aquilegia formosa* Fisch. ex DC., *Balsamorhiza deltoidea* Nutt., *Castilleja levisecta* Greenm., *Dodecatheon hendersonii* A. Gray, *Dodecatheon pulchellum* (Raf.) Merr., *Festuca roemeri* (Pavlick) E. B. Alexeev, *Gaillardia aristata* Pursh, *Micranthes integrifolia* (Hook.), *Ranunculus occidentalis* Nutt., and *Silene douglasii* Hook. were imbibed with tap water in filter paper inside a pipet washer (Thermo Scientific, Waltham, MA) for 0–24 hours (Supp. Fig. 1, Table 1, Supp. Table 1). Seeds were then placed in sterilized petri dishes on moist filter paper and underwent stratification in a dark environmental chamber at 3° C for 0–90 days (Supp. Table 1). Imbibition and stratification times, where available, were based on previous research (Krock et al. in review).

Species	Common Name	Family	Status	Other
Aquilegia formosa	Western columbine	Ranunculaceae		
Balsamorhiza deltoidea	Deltoid balsamroot	Asteraceae	G5, S2	*E. taylori* nectar
Castilleja levisecta	Golden Paintbrush	Orobanchaceae	G1, S1	*E. taylori* ovipositon
Dodecatheon hendersonii	Mosquito bills	Primulaceae		
Dodecatheon pulchellum	Darkthroat shootingstar	Primulaceae		
Festuca roemeri	Roemer's fescue	Poaceae		*P. mardon* ovipositon
Gaillardia aristata	Blanket flower	Asteraceae		
Micranthes integrifolia	Whole-leaf saxifrage	Saxifragaceae		*E. taylori* nectar
Ranunculus occidentalis	Western buttercup	Ranunculaceae		*E. taylori* nectar
Silene douglasii	Douglas campion	Caryophyllaceae		

Table 1. Ten native northwest prairie species were selected for this study. Status indicates endangered ranking globally (G) and by state (S) with lower numbers signifying most critically imperiled. "Other" shows known ecological benefit to the endangered Taylor's Checkerspot butterfly (*Euphydryas editha taylori*) or Mardon Skipper (*Polites mardon*).

Growing medium and inoculants

Medium for all treatments was Sunshine mix #2 (Sun Gro, Agawam, MA), a mix of coarse Canadian sphagnum peat moss, perlite, and dolomite. The soil was loosened and then sterilized in an autoclave at 121° C on gravity cycle for 50 min in 15 L batches. To simulate native prairie soil nutrient ratios, Apex 16-5-9 NPK Plus slow release fertilizer (J.R. Simplot, Boise, ID) was added at a ratio of 3 g per L of medium, the manufacturer's lowest recommended level.

Seeds were grown in a greenhouse with one of four arbuscular mycorrhizal fungi (AMF) inoculation treatments: 1) Native (NA) inoculant, 2) General (GE) inoculant, 3) Control (CO—no inoculant), 4) Fungicide treatment (FU—additional fungal control). The fungicide Thiophanate-methyl (dimethyl 4,4'-o-phenylenebis[3-thioallophanate]) (50% a.i., Cleary Chemical, Dayton, NJ), which has been shown to suppress mycorrhizal colonization, was added to the soil of the FU treatment at the rate of 50 mg (active

ingredient) per kg medium (dry mass) (Wilson & Williamson 2008). Contamination of controls in AMF experiments is a potential problem that can be greatly reduced by adding fungicide, however the addition may cause unwanted side effects through the elimination of other fungi present in AMF treatments, especially when a microbial wash has been applied (Wilson and Williamson 2008). We used both a non-AMF control and a fungicide control to allow comparison between the two.

Both NA and GE mycorrhizal inoculants were cultivated by Plant Health, LLC (Corvallis, OR). The NA inoculant was cultivated from the roots of eight native plants on intact prairie sites at Joint Base Lewis McChord (JBLM). Fifty grams of inoculant were added per L of growing medium in the NA and GE treatments (personal communication, B. Linderman).

Autoclaving eliminates not only mycorrhizal fungi, but all edaphic microorganisms. To simulate a variety of more-realistic soil conditions we created three soil microbial washes, with mycorrhizal spores removed, from 1) High-quality prairie (HI, >24 native plant species, JBLM), 2) Restoration prairie (RE, <10 native species, The Nature Conservancy), and 3) A control of potting soil (PO). Each microbial wash was applied to 1/3 of each mycorrhizal treatment in a full factorial design to elucidate interaction effects between AMF and soil microbial communities (Supp. Fig. 2). Following the methods of Koide and Li (1989), 3 mL deionized water per gram of field or unsterilized potting medium was combined to form a slurry, which was then filtered through a 38μm sieve to remove AMF spores. The resulting filtrate was applied at 30 mL microbial wash per L sterilized potting medium (Koide & Li 1989).

Experimental design

Each species was sown into 384 plugs (Ray Leach Cone-tainer, Tangent, OR), which had previously been sterilized with a 10% bleach solution, with two to three seeds per 107 mL plug. Plugs had been filled with growing medium that received one of four AMF treatments (NA, GE, FU, CO) and one of three microbial treatments (HI, RE, PO), for a total of 12 treatment combinations (plugs n = 3456; mycorrhizal treatment n = 864; microbial treatment n = 1,152; mycorrhizal x microbial n = 288; mycorrhizal x microbial x species n = 32). Flats were placed together in threes among 12 groups that each contained all nine species with the same AMF treatment and microbial wash; 20 cm separated groups to reduce the possibility of cross-contamination (Supp. Fig. 2). At the time of planting seeds were covered with sterilized potting soil equal to two times the thickness of the seed to reduce the possibility of cross-contamination between trays (Grman 2012). A thin layer of sterilized gravel (Gran-I-Grit starter, Mount Airy, NC) less than 3 mm deep was also added to the top of each plug to stabilize seeds and soil, and reduce bryophyte growth. Propagules were watered daily with an overhead sprinkler system and trays were rotated in the unheated greenhouse weekly.

All species were sown in February 2014, except for *F. roemeri*, which was planted in April 2014 to replace *A. formosa*, which had experienced less than 3% germination. Plugs of *A. formosa* containing seedlings were removed and remaining ungerminated plugs sown with 5–7 seeds of *F. roemeri*, selected due to its quick and consistent germination, its role as an ecosystem keystone species, and to include an additional family in the study. Any difference that occurred due to ungerminated *A. formosa* seeds was consistent throughout *F. roemeri* treatments.

Data collection and analyses

Germination data were collected weekly until, at nine weeks (when emergence had slowed and die-off begun) all plugs without seedlings were removed and the remainder thinned to the largest seedling per plug. This allowed the subsequent monitoring of survival of the one remaining individual rather than germination. Survival was tracked weekly for ten weeks and bi-monthly thereafter. Height and/or width data were collected at 16 weeks based on plant morphology, with procumbent species measured for width, upright species for height, and *R. occidentalis* for both.

At six months 60 plants from two species that had sufficient replicates (*D. hendersonii*, *M. integrifolia*) for long-term outplanting were harvested for biomass analysis. The first four plugs with visible aboveground growth were selected from each subgroup (mycorrhizal x microbial) for *M. integrifolia*. For *D. hendersonii*, which was dormant in all treatments, the first four plugs were harvested. Shoots were cut from roots at growing medium level, shoots and roots were washed with tap water, and dried at 60° C for 48 hours. Roots and shoots were weighed separately using an analytical balance (Mettler-Toledo, Switzerland).

All statistical analyses were completed using JMP Pro 11.0 software (SAS Inc., Cary, NC). Data were first analyzed for homogeneity of variance using Levene's test, and all data met requirements. Two-way ANOVA was used to analyze interaction effects of AMF and microbial wash. For height, width, and biomass, absent plants were eliminated from data. Chi-square was used for seedling emergence and survival analysis. Date of peak die-off was used for species that had experienced significant or complete die-back,

most-recent data for all others. Tukey's HSD with an alpha level of 0.05 was used to compare means.

The two mycorrhizal treatments were combined after preliminary analysis showed no statistically significant difference between NA and GE AMF inoculant for any species. As there was also no difference between the CO and FU treatments, except for in the case of *F. roemeri*, these treatments were also binned for all other species, allowing greater statistical power and simplicity of data presentation. For *F. roemeri* CO and FU data were not combined, and ANOVA of each AMF x microbial group was executed.

RESULTS

Growth

At 16 weeks, inoculation with arbuscular mycorrhizal fungi had a significant positive effect on the growth of the majority of plants tested, including *Castilleja levisecta* ($F_{[1,77]}$=5.75, p<0.02), *Dodecatheon pulchellum* ($F_{[1,292]}$=19.23, p <0.0001), *Micranthes integrifolia* ($F_{[1,351]}$=159.35, p <0.0001), *Ranunculus occidentalis* (height $F_{[1,113]}$=4.05, p <0.05, width $F_{[1,113]}$=5.15, p <0.03), and *Silene douglasii* ($F_{[1,77]}$=5.93, p <0.02) (Fig. 1). AMF inoculation did not significantly affect the growth of *Balsamorhiza deltoidea* ($F_{[1,43]}$=0.06, p=0.81), *Dodecatheon hendersonii* ($F_{[1,67]}$=2.5, p=0.19), *Festuca roemeri* (see below), or *Gaillardia aristata* ($F_{[1,39]}$=3.79, p=0.06) (Fig. 1).

There was no significant difference between the NA AMF inoculant and GE inoculant on growth at 16 weeks for any species (data not shown). There was also no difference between the CO and FU control treatments, except for on *F. roemeri*, where the FU treatment significantly enhanced growth ($F_{[2,348]}$=13.45, p<0.0001) (Fig. 2).

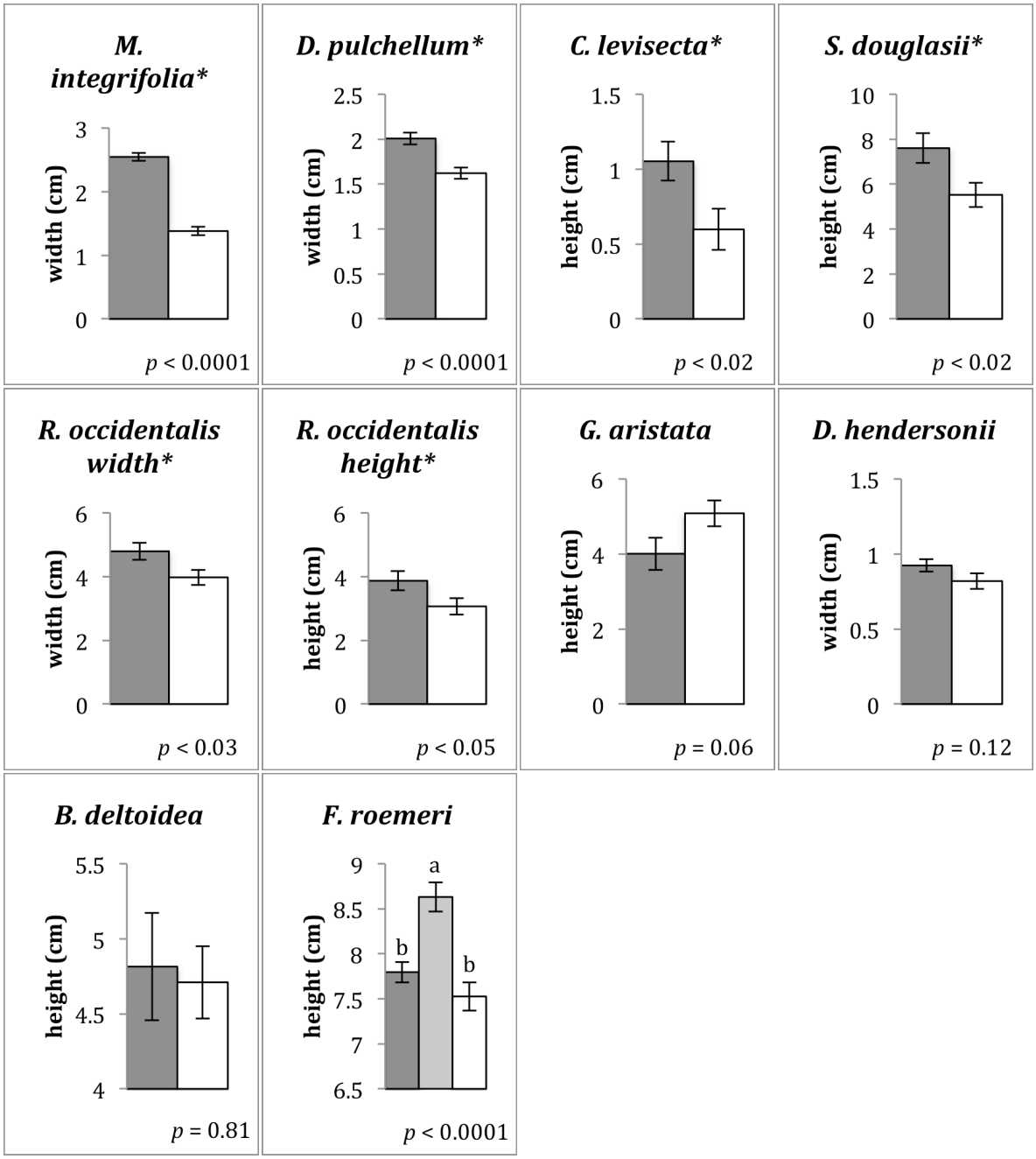

Figure 1. Growth response of seedlings to inoculation with arbuscular mycorrhizal fungi at 16 weeks. Dark gray bars show AMF treatment, white control, and light gray fungicide. Height, width, or both were measured based on species morphology. For *F. roemeri,* fungicide and control treatments were different and both are shown, lower case letters denote significant differences (Tukey's HSD) at an alpha level of 0.05. Bars show ± one standard error from the mean.
***indicates statistically significant effect**

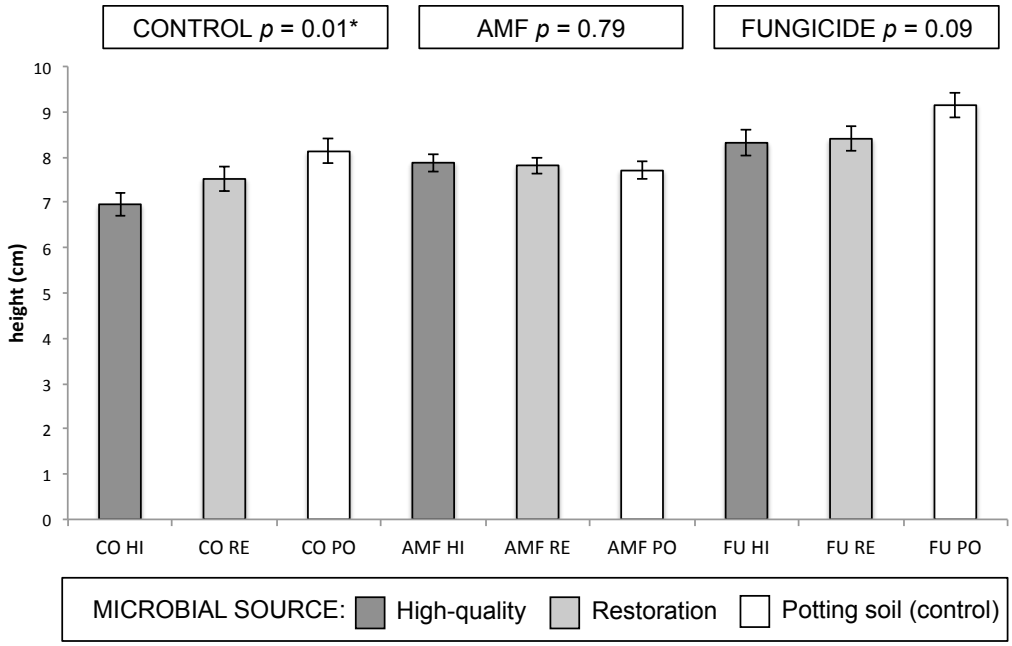

Figure 2. ANOVA showed that arbuscular mycorrhizal fungi (AMF) mediated the negative effects of microbial wash on *Festuca roemeri*. AMF treatments are grouped, CO=control, AMF=inoculant, FU=fungicide (Thiophanate-methyl), within, microbial treatments are black HI=high-quality prairie, gray RE=restoration site, white dotted PO=potting soil. Bars show ± one standard error from the mean.

Both species harvested for biomass measurements had significantly greater root biomass with AMF inoculation (*D. hendersonii* $F_{[1,44]}$=10.21, p<0.003, *M. integrifolia* $F_{[1,45]}$=29.67, p<0.0001) (Fig. 3). Shoots were only available for *M. integrifolia,* as *D. hendersonii* had entered dormancy, and were also positively affected by AMF inoculation ($F_{[1,45]}$= 20.07, p<0.0001), as was total biomass with *D. hendersonii* showing a greater than 70% increase with AMF ($F_{[1,44]}$=10.21, p<0.003) and *M. integrifolia* 189% ($F_{[1,46]}$=32.32, p<0.0001). Shoot-root ratio was not significant ($F_{[1,46]}$=0.35, p=0.56) (Fig. 4). The mass of individual *D. hendersonii* roots showed a significant correlation to week entering dormancy with longer-living shoots producing larger roots (r^2=0.35, $F_{[1,40]}$=21.27, p<0.0001) (Fig. 5).

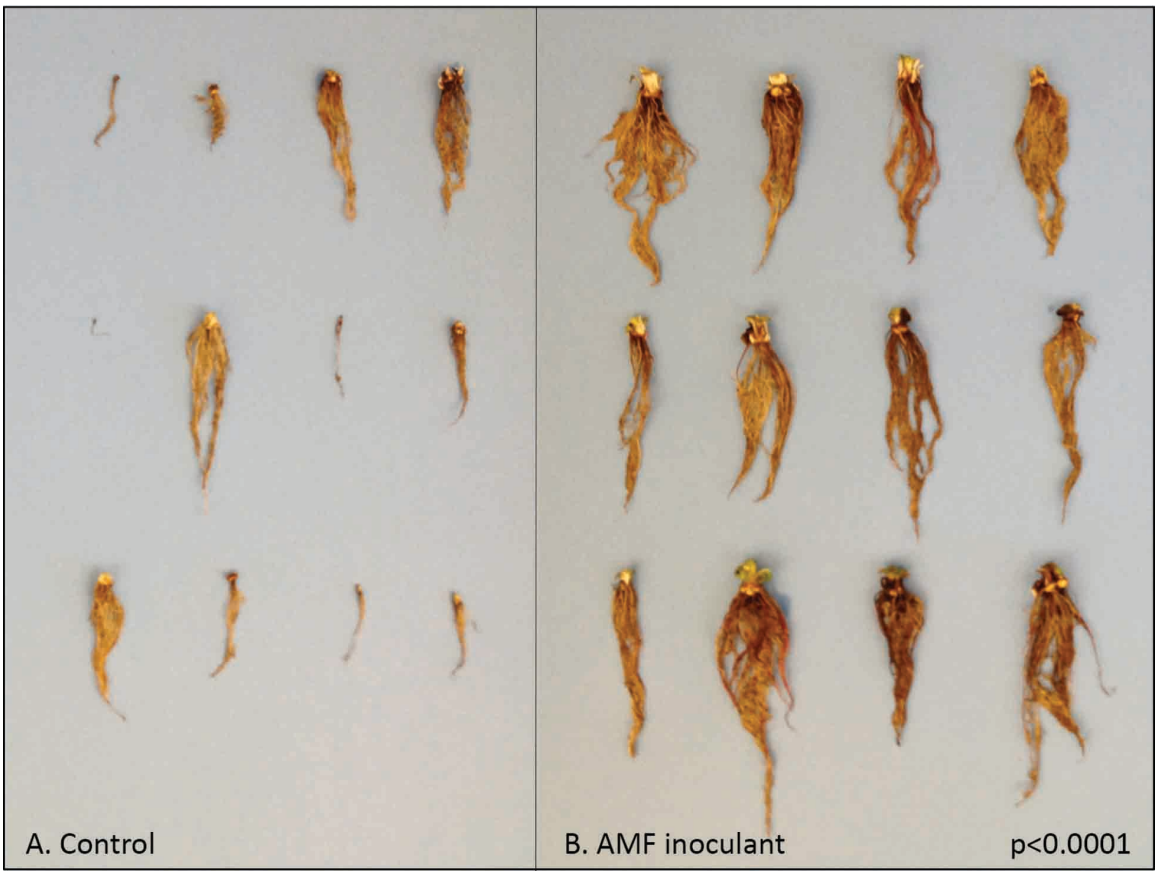

Figure 3. Roots of *Micranthes integrifolia* harvested at six months from plants A) in a nonmycorrhizal control and B) inoculated with arbuscular mycorrhizal fungi.

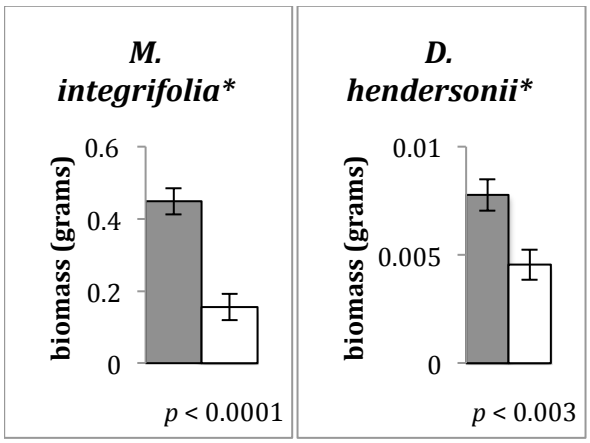

Figure 4. Belowground biomass of *Micranthes integrifolia* and *Dodecatheon henderonii* were significantly increased by inoculation with arbuscular mycorrhizal fungi (gray, control white). Bars show ± one standard error from the mean.

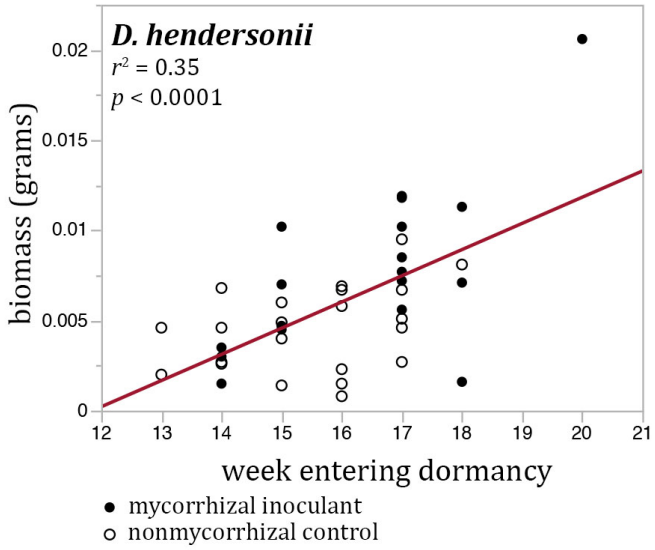

Figure 5. Correlation of root biomass and week entering dormancy for *Dodecatheon hendersonii*. Plants were harvested six months after planting. The line shows the relationship between week entering dormancy and root biomass for all treatments.

Two species showed a significant growth response to microbial inoculant with the restoration treatment (RE) suppressing *B. deltoidea* ($F_{[2,42]}$=4.51, p <0.02), and the high-quality treatment (HI) suppressing *R. occidentalis* (height $F_{[2,112]}$=6.58, p=0.002, width $F_{[2,112]}$=5.15, p=0.007) (Fig. 6). Two-way ANOVA showed significant interaction between AMF inoculant and microbial inoculant on growth for *F. roemeri* (AMF*microbial F=4.83, p<0.009) (Table 2).

Source	df	Sum of squares	F	*p*
Model	5	39.1	3.45	<0.005
Error	345	782.95		
Corrected total	350	822.06		
Mycorrhizal	1	6.02	2.65	0.1
Microbial	2	11.17	2.46	0.09
Mycorrhizal*Microbial	2	21.94	4.83	<0.009

Table 2 Results of two-way ANOVA were significant for interaction between arbuscular mycorrhizal fungi and microbial source for *Festuca roemeri.*

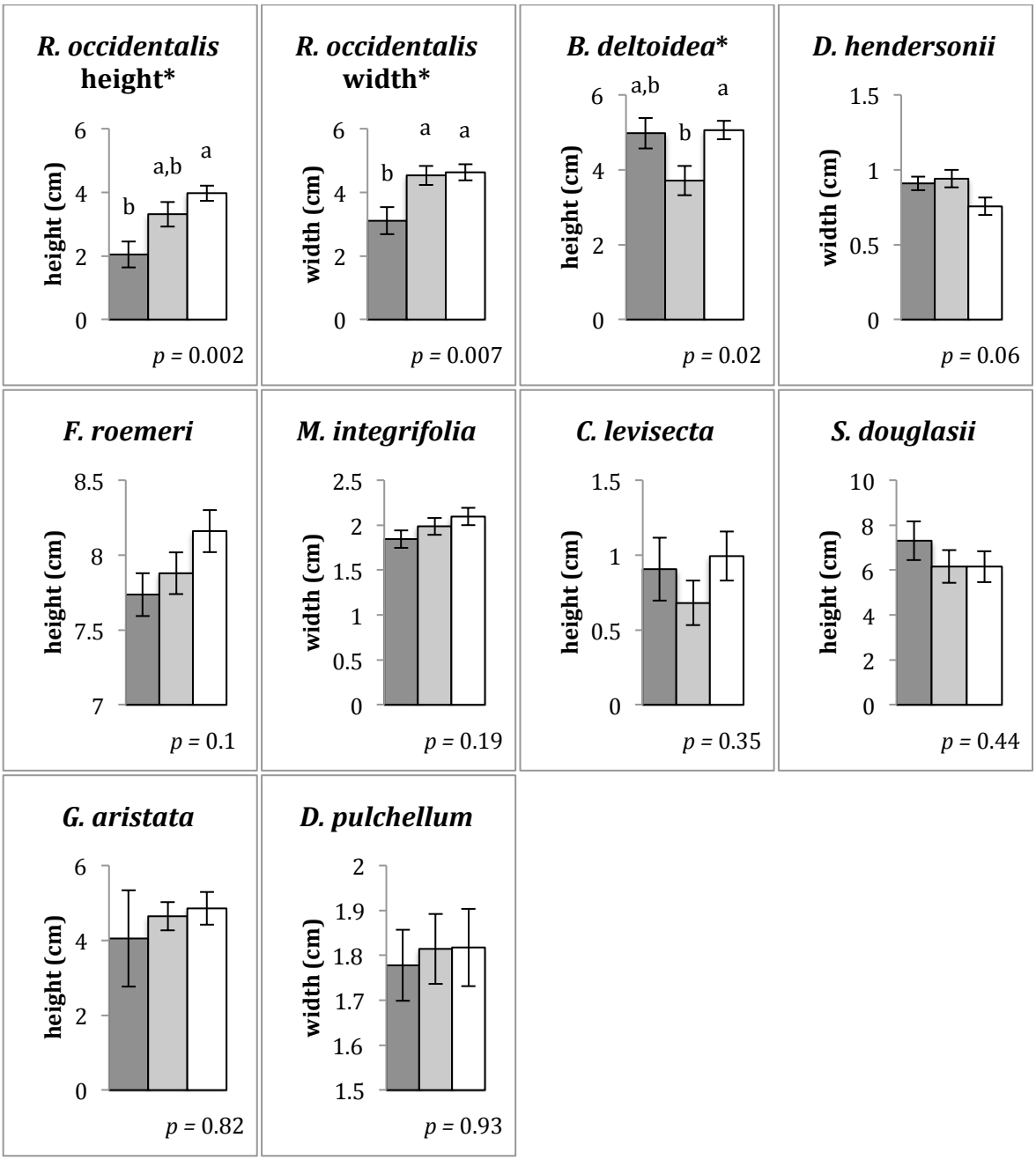

Figure 6. Growth response of seedlings at 16 weeks to inoculation with three microbial washes (high-quality prairie source dark gray, restoration gray, white control). Height, width, or both were measured based on species morphology. Lower case letters denote significant differences (Tukey's HSD) at an alpha level of 0.05, bars show ± one standard error from the mean.
***indicates statistically significant result**

Within *F. roemeri* mycorrhizal treatments there was a significant difference for microbial wash within the CO treatment ($F_{[2,86]}$=4.43, p=0.01) but not the AMF ($F_{[2,175]}$=0.24, p=0.79) or FU treatment ($F_{[2,81]}$=2.4, p=0.09) (Fig. 2).

Emergence and Survival

Seedling emergence was unaffected by AMF for all but *B. deltoidea,* which showed a 7–8% decrease in emergence with inoculation ($X^2_{[1,384]}$=4.83, p<0.03). Field microbial inoculant tended to have a negative effect on emergence (Table 3a). This was observed for the HI inoculant in *B. deltoidea* ($X^2_{[2,384]}$=14.65, p=0.0007), *C. levisecta* ($X^2_{[2,384]}$=14.01, p=0.0009), *G. aristata* ($X^2_{[2,384]}$=13.63, p=0.001), and *R. occidentalis* ($X^2_{[2,384]}$=22.4, p<0.0001), though there was a positive effect on *D. pulchellum* ($X^2_{[2,384]}$=16, p=0.0003). The RE inoculant negatively affected *B. deltoidea* ($X^2_{[2,384]}$=14.65, p=0.0007), and *R. occidentalis* ($X^2_{[2,384]}$=22.4, p<0.0001), but had a positive effect on *D. pulchellum* ($X^2_{[2,384]}$=16, p=0.0003) and *G. aristata* ($X^2_{[2,384]}$=13.63, p=0.001).

Survival of plants overall was significantly positively affected by AMF inoculation ($X^2_{[1,1529]}$=18.5, p<0.0001). AMF did not have a negative effect on the survival of any species and positively affected *C. levisecta* (at week 24 $X^2_{[1,85]}$=16.48, p<0.0001), *D. hendersonii* (at week 15 $X^2_{[1,178]}$=0.003), *M. integrifolia* (at week 28 $X^2_{[1,314]}$=31.35, p<0.0001), and *R. occidentalis* (at week 28 $X^2_{[1,116]}$=3.78, p<0.05). Microbial inoculation only affected the survival of *F. roemeri*, with the HI inoculant having a negative effect (week $X^2_{[2,356]}$=6.17, p<0.05).

	Balsamorrhiza deltoidea	*Castilleja levisecta*	*Dodecatheon hendersonii*	*Dodecatheon pulchellum*	*Festuca roemeri*	*Gaillardia aristata*	*Micranthes integrifolia*	*Ranuculus occidentalis*	*Silene douglasii*
a) Seedling Emergence									
AMF	-	0	0	0	0	0	0	0	0
p	<0.03	0.7	>0.05	0.7	*	0.46		0.08	0.13
HI micro	-	-	0	+	0	-	0	-	0
RE micro	-	0	0	+	0	+	0	-	0
p	<0.001	<0.001	0.2	<0.001	*	0.001	0.56	<0.0001	0.1
b) Survival									
week	19	24	15	28	28	28	28	28	28
AMF	0	+	+	0	0	0	+	+	0
p	1	<0.0001	<0.003	0.06	0.63	0.76	<0.0001	0.05	0.83
HI micro	0	0	0	0	-	0	0	0	0
RE micro	0	0	0	0	0	0	0	0	0
p	0.23	0.14	0.81	0.81	<0.05	0.17	0.43	0.6	0.27

Table 3. Chi-square analysis of the effects of arbuscular mycorrhizal fungi (AMF) inoculation and microbial wash on a) seedling emergence and b) survival. Effect of inoculant is indicated as + (positive) or – (negative) where results are statistically significant, or 0 where there was no significant effect. HI = microbial wash derived from high-quality intact prairie RE = microbial wash from a lower-quality prairie site undergoing restoration. Date of peak die-off was used in survival analysis for species that had experienced significant or complete mortality, most-recent data for all others.
*** *F. roemeri* experienced 100% emergence in all treatments**

DISCUSSION

AMF Inoculation

Overall, inoculation with arbuscular mycorrhizal fungi (AMF) had a positive effect on the growth and survival of the prairie species studied. As none of the species tested had a negative growth or survival response to inoculation, and AMF will be present in outplanting sites, inoculating all prairie species raised for outplanting without conducting further species-specific trials, which can be expensive and time-consuming, would be justifiable for restoration purposes.

We had hypothesized that plants grown with a native (NA) AMF culture would be more likely to experience positive responses to inoculation, but found that a general (GE) commercially available culture was equally beneficial, producing the same results for all species. Acquiring, growing, and maintaining native field AMF cultures can be problematic because: 1) field inoculum may be unavailable, 2) it is unknown whether AMF field communities are representative of historical counterparts, and 3) for a variety of reasons, AMF cultures tend to change over time (IJdo et al. 2011). The latter 2 cases are likely true for this study, as individual AMF identity within the NA and GE cultures has not yet been established, or the advantages of the NA culture may not be quantifiable over the short term. Because it has been well-established that different plant and AMF species, and even genotypes interact differently, and because introducing new nonnative strains of AMF may be of concern (Schwartz et al. 2006), we believe that more research on the development, maintenance, and effect of native AMF cultures for restoration is needed, and that when possible practitioners should use native cultures (Anderson and Roberts 1993, Klironomos 2003).

The significant positive response of over half of the species tested to AMF inoculum after only six months indicates that AMF have likely been an important part of the WPG ecosystem, allowing increased access to nutrients in gravelly glacial outwash soils, and influencing the evolutionary strategy of plants that evolved there since the last ice age. Vogelsang and Bever (2009) found that in California grasslands non-native plants, which tend to depend less on AMF, cause mycorrhizal densities in soil to decrease, creating less hospitable conditions for native forbs and a feedback loop that increases invasion. A recent study on WPG prairies found that *R. occidentalis* roots were three times more colonized by AMF in a high-quality remnant than in a more heavily degraded restoration area (Block & Hamman, unpublished data). On sites that have already been heavily affected by altered land use histories and non-native plant invasion (especially shrubs and trees with ectomycorrhizal associates), AMF communities are likely already in a degraded state and the reintroduction of mycorrhizal fungi may be beneficial regardless of origin. However, managers should take more caution in outplanting seedlings with potentially non-native AMF into intact ecosystems, though this suggestion is based in caution rather than empirical evidence (Schwartz et al. 2006). Mycorrhizal response of natives, which is often high, versus invasives, should be taken into consideration before inoculating seedlings (Wilson & Hartnett 1998).

The only negative response to AMF found was a 7–8% decrease in *Balsamorrhiza deltoidea* seedling emergence. Germination was problematic for several of the species in this study, and may have been due to uncharacteristically cold conditions that reached less than –9° C the first week after planting (NOAA 2014), the use of seeds that were ~3 years old, or that some species had been selected for general study because of the desire

to improve the historically low germination rates. AMF are not generally thought to affect seedling emergence, which was the case for eight of the nine species studied, though Richter and Stutz found that inoculation positively affected *Sporobolus wrightii* (2002). Poor germination rates combined with the goal of producing sufficient material for a long-term outplanting study hindered the harvesting of most species for biomass data, and we recommend that practitioners interested in studying AMF response overseed at a rate of more than two to three seeds per plug for species that are known to have low emergence rates. Heavily overseeding, however, should be avoided, as it may mask results by producing germinants in every plug, as was seen with *F. roemeri*—it was the variability in germination in this study that allowed us to analyze the negative effect of field soil microbial washes on emergence (Table 3a).

Soil Microbial Wash

It is common practice in AMF experiments to reintegrate soil microbial communities (sans AMF) back into sterilized media, and it has generally been found that plants respond more positively to AMF with greater rhizosphere community complexity (Hoeksema et al. 2010). We therefore expected the microbial wash that we derived from a high-quality intact prairie site to have a positive interaction with AMF and on the growth and survival of plants, but found instead that the HI and RE treatments tended to repress plant growth, though reactions were species-specific and mostly not significant (Fig. 2). The effect of field inoculant on seedling emergence, however, showed a negative trend (Table 3a). A likely explanation for these effects is that field soils contained pathogens that had differing species-based effects, though other causes cannot be

excluded. While the three washes likely contained different concentrations of microorganisms, these concentrations were representative of sites and consistent within treatments.

If pathogens were the main factor, plant survival was unaffected except for in *F. roemeri*, and the repressive trend on growth could actually lead to increased field survival as pathogen inoculated plants may have developed defenses under favorable greenhouse conditions while PO plants will first be exposed in the competitive field environment (Table 3b). The negative effect of field microbial inoculant on seedling emergence for four species is concerning, suggesting that even the most intact remnant prairie sites may be inhospitable places for native regeneration. Our data indicate that practitioners should not apply microbial washes in the greenhouse until several weeks after seedlings have emerged, and that the use of whole soil for AMF inoculation may be problematic in some ecosystems even though it has been shown to have greater efficacy than cultured ones in others (Rowe et al. 2007).

Species-Specific Responses

Dodecatheon hendersonii is an ephemeral species, one of the first flowers to appear on WPG prairies in the spring and also one of the first to die back. During the first growing season the species produces only cotyledons aboveground, concentrating energy into its fleshy lateral root. At four months a significantly higher number of non-mycorrhizal plants had entered dormancy than those treated with AMF (Table 3b). Unfortunately we had not measured aboveground growth before the majority of this die-off occurred, which likely skewed growth data through the measurement of only the few most vigorous plants

in the control treatments; if measurements had been taken earlier it is likely that growth would have been significantly positively influenced by AMF, as well (Fig. 1). We hypothesize that the extension of plants' photosynthetically active phase would lead to larger, more-developed belowground parts, and increased long-term survival. Data on individual plant presence were collected weekly, allowing an analysis of the relationship between week entering dormancy and biomass of belowground growth in plugs that were harvested two months later. A strong relationship was found between week entering dormancy and belowground biomass, with increasing aboveground survival time correlated with larger root biomass (Fig. 6). In the field, plants that experience a longer growth period may be more likely to experience pollination and establish viable populations. We are currently unaware of other studies on the relationship between AMF and plant phenology.

Another unexpected finding was the positive effect of AMF on the growth and survival of *Castilleja levisecta*, a facultative hemiparasite. Parasitic plants produce haustoria, physical structures that penetrate neighboring root systems, and have long been considered some of the few non-mycorrhizal plants, however recent studies have shown that AMF can colonize the roots of these species (Atsatt, Peter R. 1973, de Vega et al. 2010, Li et al. 2012). Parasitic plants have been observed to benefit from the AMF status of their hosts, and may utilize the pathways that mediate AMF recognition and colonization in host plants, causing hosts with higher AMF dependency to be more receptive to parasites (Davies & Graves 1998, Fernández-Aparicio et al. 2010). While the increased growth and survival of *C. levisecta* with AMF and no host is both surprising and promising, it is unclear whether these benefits will continue under field conditions.

Li et al. (2012) found that a hemiparasite grown with AMF experienced reduced haustoria formation and repressed growth after outplanting. This study provides an excellent opportunity to further study the relationships between AMF, parasitic plants, and hosts.

F. roemeri showed an especially interesting response to our treatments that supports the theory that field microbial washes carried pathogens. This grass was the only species for which the FU treatment, designed as a secondary control for AMF contamination, had a significantly different effect than the CO treatment (Fig. 1). Fungicide enhanced the growth of *F. roemeri*, likely by killing rusts (*puccinia* spp.), which the plant is known to be susceptible to (Darris 2005), in the microbial wash. There was no significant effect of microbial wash within the FU treatment, while the CO treatment was negatively affected by the HI edaphic community (Fig. 2). Interestingly, AMF inoculated plants were also not affected by microbial wash, though seedlings were smaller than those that received fungicide, likely due to carbon allocation to AMF (Smith & Read 2008). This indicates that AMF mediated the negative effects of the microbial pathogen, though with a greater overall growth, but not survival, cost than fungicide.

Conclusion

These results indicate that use of AMF in the propagation of native species for outplanting is likely to produce a greater number of seedlings overall, and further that these seedlings will generally have larger above- and below-ground structures. Practitioners should keep in mind that even where AMF inoculation does not positively affect growth, it can act as a sort of "insurance policy"; it is advantageous for plants to

give small regular amounts of carbon, which can reduce short-term resources, for a possible future payout if conditions become adverse, such as can occur with pathogens or drought (Ferrazzano & Williamson 2013).

Upon outplanting, greenhouse propagules will be exposed to conditions that include competition with neighboring native and non-native plants, exposure to pathogens and other microorganisms, soil with different nutritional and physical characteristics, herbivory from new species, and more-intense temperature and water variability. The fact that plants are often linked by roots into complex mycorrhizal networks with nutrients and signals transferred between plants may provide additional benefits to plants in the field (van der Heijden & Horton, 2009). These interacting factors, and changing environmental conditions, make it difficult to propagate greenhouse seedlings that will be well-adapted to the field, and it is therefore important for practitioners to rethink traditional horticultural techniques when producing plants for restoration.

After six months of greenhouse study, it was found that AMF inoculation successfully produced a greater number of plants with increased robustness, can extend phenology, cause growth and survival benefits even in a hemiparasite, and mediate the effects of soil pathogens. While this study focused on plants from one ecosystem, we hope that results will encourage practitioners worldwide to try growing seedlings with AMF, especially in areas characterized by low-nutrient soil or non-native invasion. Seedlings produced in this experiment were outplanted in fall of 2014, and we anticipate that field results collected over the long term will provide further answers to the questions raised in this study.

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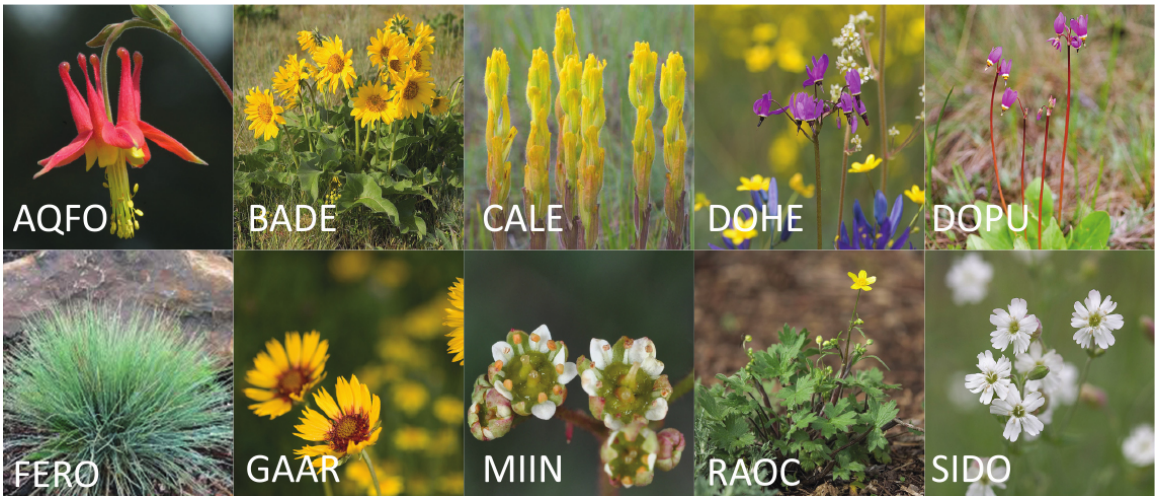
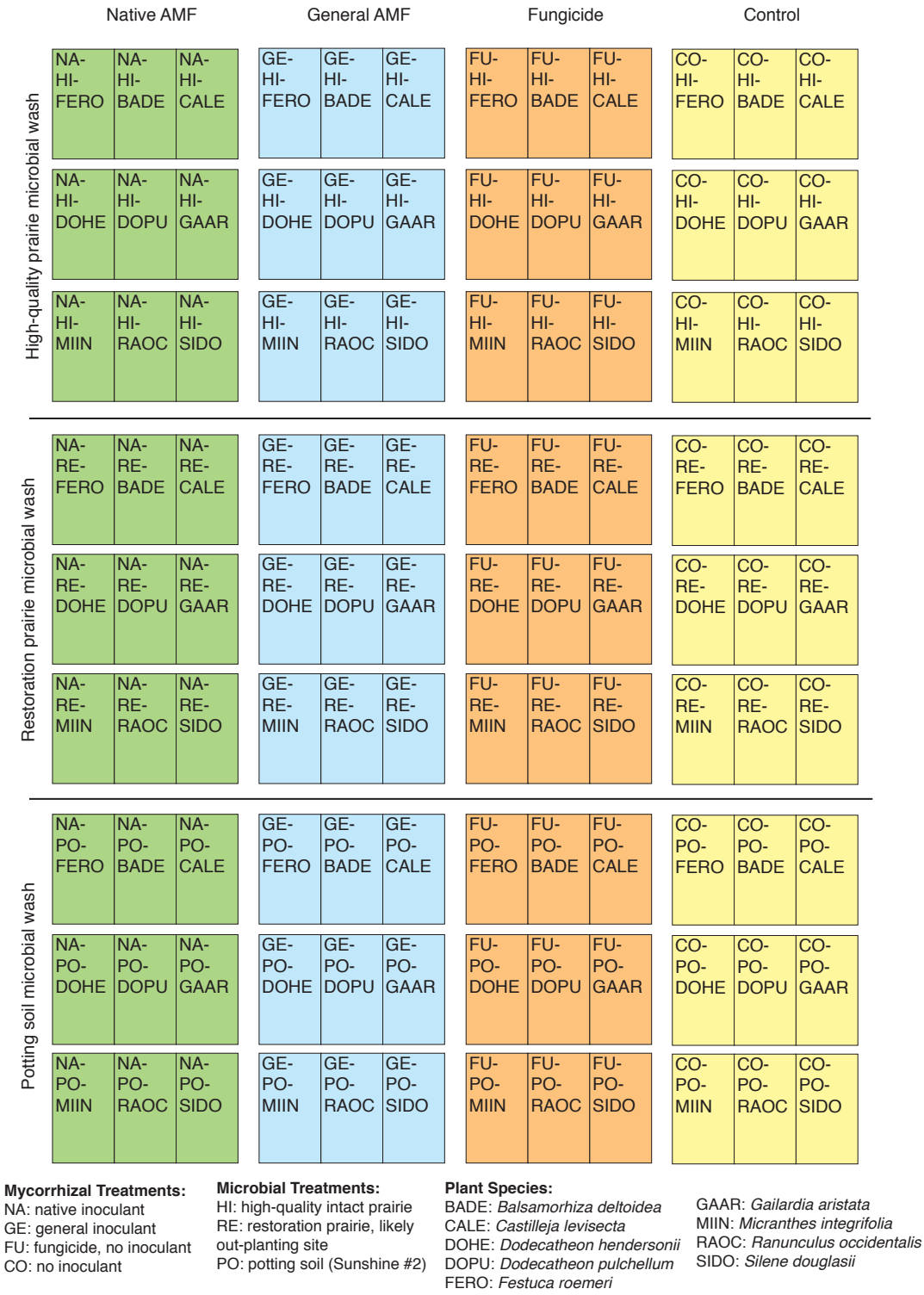
SUPPLEMENTAL FIGURES AND TABLES



Supplemental Figure 1. Perennial Pacific Northwest Willamette Valley–Puget Trough–Georgia Basin species studied. From left to right, first row: *Aquilegia formosa, Balsamorhiza deltoidea, Castilleja levisecta, Dodecatheon hendersonii, Dodecatheon pulchellum.* Second row: *Festuca roemeri, Gaillardia aristata, Micranthes integrifolia, Ranunculus occidentalis, Silene douglasii.* Photographs used with permission of Rod Gilbert.

Species	Imbibition time (hours)	Stratification time (days)	Imbibition date	Stratification dates
Aquilegia formosa	24	60	11/30/13	12/1/13–1/30/14
Balsamorrhiza deltoidea	8	30	12/30/13	12/30/13–1/30/14
Castilleja levisecta	12	15	1/14/13	1/14/14–1/30/14
Dodecatheon hendersonii	24	15	1/14/13	1/14/14–1/30/14
Dodecatheon pulchellum	24	60	11/30/13	12/1/13–1/30/14
*Festuca roemeri**	0	0	0	0
Gaillardia aristata	8	90	10/30/13	10/30/2013–1/30/14
*Micranthes integrifolia**	12	30	12/30/13	12/30/13–1/30/14
Ranunculus occidentalis	12	0	1/29/13	0
Silene douglasii	24	60	11/30/13	12/1/13–1/30/14

Supplemental Table 1. Seeds underwent imbibition (soaking) and stratification (dark storage at 3° C) based on previously established protocols (Krock et al. in review). **Micranthes integrifolia* and *Festuca roemeri* were added to this study without established imbibition or stratification times.



Supplemental Figure 2. Treatments were arranged for each tray to contain one mycorrhizal treatment, one microbial treatment, and three species. Rectangles represent one tray divided into three sections, each containing 32 plugs of three species, with 96 plugs per tray.

Restoration and Its Discontents:

A Critical Analysis of Ethics and Practice in Applied Ecology

INTRODUCTION

Studying the effects of mycorrhizal and microbial organisms on native plants, grown for outplanting to rare prairie sites, is heavily rooted in the fields of conservation biology and restoration ecology, as are the values and assumptions behind their practice. Restoration ecology is a fairly new and highly interdisciplinary area of study with still-developing philosophies and praxis. Looking critically at some of the problems with both the underlying philosophies and evolving methods of restoration is especially important as the goal of research in this field is usually less to inform basic science than to affect practice. And the stakes are huge—restoration could, as E. O. Wilson believes, be " . . . the means to end the great extinction spasm. The next century will . . . be the era of restoration in ecology" (Wilson 1992). Unfortunately, practitioners could instead be creating large new problems, encouraging systems that require constant human intervention, undermining conservation efforts, or simply wasting resources.

The term "restoration" has an inherently positive connotation; it makes us think of broken things repaired, artworks and buildings returned to their original splendor, and when combined with "ecology," of the possibility of returning nature to a pre-human state. The Society for Ecological Restoration defines it as " . . . the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed," (Clewell et al. 2004 p. 3, Wilson 1992). Yet restoration ecology has also received deserved criticism, and many think that the seemingly unimpeachable ethical foundations on which it stands are actually quite shaky.

AUTHENTICITY

In "Faking Nature," an early critique, Robert Elliot (1982) compares environmental restoration to the forgery of great works of art. Elliot argues that whether or not we are aware of the deception, something of value is lost in the copy, and that our perceived value of a work of art or piece of nature is tied up not only in the final result, but in our assumptions about the process that led to the product. In the case of nature, the implication is that ecosystems that have come into their current state without human intervention are more authentic and of greater value than landscapes that have not experienced human alteration.

Eric Katz (1997) builds on Elliot's ideas in "The Big Lie: Human Restoration of Nature," warning of the dangers of believing that humanity has the ability to restore or repair the natural environment. Like Elliot, Katz makes distinctions between the natural and artificial, but develops a more complex argument, acknowledging that the distinction exists on a continuum rather than as a dichotomy, and that "When we thus judge natural objects, and evaluate them more highly than artifacts, we are focusing on the extent of their independence from human domination (p. 396)."

Two main responses to Elliot's and Katz's arguments have developed within the fields of environmental ethics and restoration ecology. The first addresses the difficulties and ethical problems with separating the concepts of natural from artificial, human from environment, and attaching value to these. Doing so is not only difficult, potentially racist and classist, but may ultimately hinder the formation of a viable land ethic that leads to positive environmental change. The second is that Katz and Elliot's arguments tend to focus on taking pristine environments, destroying them, and then restoring them, a

process that Andrew Light (Light 2000) terms "malicious restoration." Light argues that this practice is irrelevant to most ecological restoration projects, which work with landscapes that were altered by humans, and have existed in that altered state, with no intentional destruction occuring.

HUMANS, NATURE, AND LINES

Elliot's line of demarcation between natural and artificial, whether it ever existed at all, has grown fuzzier for environmental thinkers in the past few decades, perhaps receiving its final blow in the scientific acceptance that we have entered a new geologic era, the anthropocene, where human-caused climate change is affecting even Antarctica, the one continent that appears never to have had major human settlements (Crutzen 2006). This theoretical line has long been debated by philosophers—are the crops and animals we grow unnatural? Are they then artifice? Katz would likely point out that these processes involve domination of nature and are therefore unnatural.

Indigenous people

Indigenous people, and their role in shaping environments, are awkardly missing from Elliot's and Katz's arguments. Richard White addresses some of the problems with dichotomous environmentalist thinking eloquently:

> We are pious toward Indian peoples, but we don't take them seriously; we don't credit them with the capacity to make changes. Whites regularly grant certain nonwhites a 'spiritual' or 'traditional' knowledge that is timeless. It is not something gained through work or labor; it is not contingent knowledge in a contingent world. In North America, whites are the bearers of environmental original sin, because whites alone are recognized as laboring. But whites are thus also, by the same token, the only real bearers of history. This is why our flattery (for it is usually intended to be such) of 'simpler' peoples is an act of such immense condescension. For in a modern world defined by change, whites are portrayed as the only beings who make a difference (1996, p. 175).

Evidence of ancient and extensive indigenous environmental manipulation using hundreds of strategies is becoming increasingly well-established in the scientific literature. Even areas deep in the Amazon, originally thought to be truly "untouched" show evidence of vibrant anthropogenic land management (Posey 1985). Overwhelming evidence from a wide variety of disciplines indicates that Pacific Northwest prairies and oak savannas were shaped and maintained by the intentional use of fire for the last several thousand years (Cooper and Suckley 1859, Boyd 1999, Walsh et al. 2010, Sprenger and Dunwiddie 2011). Admitting the role of ancient humans in ecosystem creation and maintenance, however, is damaging to Katz and Elliot's arguments. If nature is defined as authentic only when it remains in an untouched state, finding thousands of years of fingerprints in nearly every corner and crevice of our world leads to the earth-shattering conclusion that authenticity does not in fact, exist. Accepting that humans have left marks everywhere, but belittling the importance of indigenous fingerprints is both condescending and inaccurate.

Work

It is important to note that only within the last century or so has the view that human-affected environments may be less valuable than "natural" ones existed to any significant degree within Western culture (Worster 1994). Rather, wilderness has historically been seen as a force to be feared and tamed, with the pastoral landscape, controlled by humans for the creation of food, or the well-ordered flower garden, idyllic. Landscapes that represented human dominance, food productivity, and labor, were instead seen as valuable.

The “leave no trace” ethic of Katz, Elliot, and many environmentalists, is valuable as a conservation tool, but problematic as a paradigm. This is not only because of its exclusion of indigenous people and their influence on the land, but the exclusion of all people and their labor (White 1996). Demarcating land as either for conservation *or* for humans may cause us to lose some of our last real connections with natural land. White argues that humans have long formed connection to nature, and a subsequent understanding of its value and importance through labor, while modern environmentalists encourage the formation of this relationship through recreation (1996). He does caution, however, that physical labor in nature has not historically prevented it from harm. Rather, enhancing the dichotomous line suggested by philosophers such as Elliot encourages an unprecedented divorce between nature and work—one that escalates political divides and alienates people from natural environments.

Our current culture, with its swelling population and economy focused on growth, requires the setting aside of land for strict conservation. But by focusing on demarcating and defending the line between humans and nature, rather than acknowledging the blurry areas that dominate the landscape when one steps back, environmentalists are also widening divides, and abandoning huge swaths of land that could have been used for conservation purposes. Failing to see value in land that has been affected by humans and their labor, the lands that have and will continue to make human existence possible, is a serious mistake, and one that makes all but the most dedicated primativists into hypocrites, and some argue, gives environmentalism itself a bad name.

MALICIOUS RESTORATION

The second rebuttal to Katz and Elliot's qualms with the "restoration hypothesis" is that they focus on what Light calls "malicious restorations (Light 2000)." Elliot begins "Faking Nature" with a hypothetical case in which a company wants to mine a beach for a particular mineral, which will involve destroying the naturally occurring dunes and vegetation on the site. However, the company expresses its desire and willingness to return the beach to its original state after the minerals have been extracted. Light argues that this sort of malicious restoration should be ethically considered separately from "benevolent " restorations, which are carried out to fix harm that has already occurred, and are not a justification for destructive behavior. There are problems with Light's stance, including that unfortunately, malicious restoration is currently a common practice, that even benevolent restoration can cause harm, and that it can be both difficult and problematic to draw lines between the two. In fact, both of these types of restoration utilize much of the same science, and stem from the same logic, the belief that humans are in fact capable of successfully "fixing" nature (Katz 1997)

Katz, writing later than Elliot, leaves a little bit more possibility for Light's benevolent restoration:

> I believe, for example, that Exxon should attempt to clean up and restore the Alaskan waterways and land that was harmed by its corporate negligence. The point of my argument here is that we must not misunderstand what we humans are doing when we attempt to restore or repair natural areas. We are not restoring nature: we are not making it whole and healthy again. Nature restoration is a compromise; it should not be a basic policy goal. It is a policy that makes the best of a bad situation; it cleans up our mess. We are putting a piece of furniture over the stain in the carpet, for it provides a better appearance. As a matter of policy, however, it would be much more significant to prevent the causes of the stains (1997, p. 396).

Both Katz and Elliot fear that an acceptance of the belief that humans can recreate nature and that what is recreated is of equal value to the original, will lead to thought and policy that endanger conservation efforts; if we can fix damage that we cause, or effectively recreate ecosystems in more convenient places, why should we worry about harm?

Malicious restoration has indeed worked its way into current environmental policy and practice. Legalization officially occurred in the United States in 1993 when the Clinton administration approved support for the use of "mitigation banks," and the US Environmental Protection Agency and Department of the Army issued guidelines for their establishment and use (Department of the Army, Corps of Engineers Corps of et al. 1995). These policy changes, intented to make conservation under the Endangered Species Act function within a free-market capitalist system, has validated Elliot and Katz's fears (Bayon et al. 2012). Wetlands can be filled and developed if constructed wetlands are installed elsewhere, forests logged if they are replanted. Development mitigation allows legal environmental destruction, even where it may otherwise have been illegal, by allowing companies to purchase environmental credits in the form of "projects that restor[e], creat[e], enhanc[e], and in exceptional circumstances, preserv[e]" to mitigate the debit that the original develompent project caused (Department of the Army, Corps of Engineers Corps of et al. 1995). Restoration organizations, including the Center for Natural Lands Management, through which this thesis work has been done, benefit from these sorts of policies, which privatize conservation and make restoration financially viable.

I believe that we are still at a point in history where, philosophically and scientifically, re-created nature is not considered to be equally exchangeable with more-

natural environments, even though legally they may be treated as equivalent (US DOD et al. 2005). I use the term more-natural here because I don't agree with drawing a strong line between humans and nature, but do see a major difference between parcels that have been subject to restoration activities, and ones that those restoration parcels are emulating. Ironically, restoration ecologists are likely among those who best understand this difference.

In the last decade, careful work on the development of locally adapted yet genetically diverse native seed materials, and the extrapolation of appropriate "seed zones" within which to source materials has been developing (Bower et al. 2014). Within species, genotypes are often adapted to very local climatic and ecological variations, and there is often great diversity even within local genotypes (Rogers & Montalvo 2004). Historically, the amounts of native seed needed for restoration projects has not been readily available, and seed materials that are locally maladapted, genetically homogenous, and selected for by cultivation practices have been introduced to restoration sites, often spreading their traits through gene flow with wild plants (Bower et al. 2014). As a result, those collecting native seed from wild populations based on current understanding do their best to avoid sites where restoration materials may have previously been introduced (US BLM 2014). Restorationists are quite aware that the practice's techniques do not yet come close to actually recreating the authenticity of more-natural sites. The law, unfortunately, does not seem to have the same scruples.

Will further blurring the lines between restored and more-natural landscapes lower their value and increase confidence that humans can successfully recreate nature? Will developing strategies that improve the success of restoration efforts lead to policies

that promote malicious restoration rather than conservation? Should restorationists who have benevolent intentions worry that the techniques they develop may be put to use in unethical ways? Restoration ecology is coming of age, and will need to answer these Oppenheimer's dilemma questions that have sprouted from the seeds sown by Elliot and Katz.

PROBLEMS WITH BENEVOLENT RESTORATION

What will E. O. Wilson's era of restoration in ecology actually look like? Some, such as Peter Del Tredici, a plant ecologist who wrote "Neocreationism and the Illusion of Ecological Restoration," theorizes that it is most likely to resemble yard work—and a lot of it:

> What's striking about this so-called restoration process is that it looks an awful lot like gardening, with its ongoing need for planting and weeding. Call it what you will, but anyone who has ever worked in the garden knows that planting and weeding are endless. So the question becomes: Is "landscape restoration" really just gardening dressed up with jargon to simulate ecology, or is it based on scientific theories with testable hypotheses? To put it another way: Can we put the invasive species genie back in the bottle, or are we looking at a future in which nature itself becomes a cultivated entity (Del Tredici, 2004, p. 2)?

While Richard White might appreciate the implications of a large-scale reintegration of human labor into nature on some level, it is a problematic prospect. According to Katz, "A policy of domination subverts both nature and human existence; it denies both the cultural and natural realization of individual good, human and nonhuman. Liberation from all forms of domination is thus the chief goal of any ethical or political system (1997, p. 396)." Restoration is thus solving the problems of human domination through further domination, a proposal that it is not only theoretically problematic, but has led to

real problems, such as the introduction of inappropriate "native" plant genotypes (Bower et al. 2014).

Is restoration, as Del Tredici suggests by mentioning neo-creationism in his title, an attempt to play god, fighting nature itself? Are we turning ourselves into backward-looking juvenile deities, still experimenting with how to act, but convinced that this time, at least, our intentions are good? This comes down to the most difficult social and environmental question of our time: Can we create change within our existing system, or will current praxis need to be altered or disassembled on a large scale before a sustainable future can be reached? Restoration ecology is a child of the current framework, solving small problems within the limits of the system, while Elliot and Katz advocate a major shift.

Invasive species and dates

Elliot and Katz are right on some levels, but they are also not ecologists or biologists, they are philosophers. Invasive species, a product of human travel and globalization, have become a nearly insurmountable problem in many ecosystems (Pimentel et al. 2005). In terrestrial environments non-native plants that have traveled with people, escaped from gardens, or been intentionally introduced often become invasive, dominating the landscape and outcompeting natives. Ecological checks and balances that kept these plants under control in their original habitats are absent from their new ones, allowing them to spread without restrictions, shading out natives, and leaving ecosystems with greatly reduced diversity, sometimes to the point of monoculture, and even soil microbial communities and biogeochemical cycles are altered. Plant invasions can lead to the

extinction not only of native autotrophs, through competition and hybridization, but the animals that have evolved with native plant species and depend on them for food and habitat. It is estimated that in the United States there are more than 50,000 foreign species, that 42% of the species on threatened or endangered species lists are at risk primarily because of invaders, and that associated economic costs are in excess of $120 billion per year (Pimentel et al. 2005).

Many restoration ecologists have had to redefine their goals due to the invasive species problem, and the exorbitant costs (and futility), associated with eradicating them. Historically, the goal of restoration in the Western hemisphere has been to return ecosystems to their imagined pre-Columbian state. However, this goal is problematic not only on practical, but social and philosophical levels.

First, it is very difficult to know with any real certainty what pre-Columbian ecosystems looked like, partly because landscapes were managed by indigenous people. Even first explorers' accounts are not always accurate, for example, in the Pacific Northwest the spread of disease with population effects more devastating than Europe's black plague had reached indigenous people in the 1770s, drastically altering land management practices long before explorers reached the area (Boyd 1999).

Second, looking at time-scales of thousands of years shows great fluctuation and variation in vegetation type, driven by changes in climate. In the Pacific Northwest, three distinct climatic eras have occurred since the last glacial maximum ended 13,900 years ago, with our modern climate and its associated vegetation only establishing 3,900 years ago (Whitlock & Bartlein 1997). In fact, the vegetational communities of the early and late Holocene were driven by a warmer, drier climate than the modern era, and may

better represent the "natural" vegetation that should occur with global warming. Attempting to hold ecosystems at a chosen point in time also violates data showing that the only constant over time has been change, caused by both human and other sources.

Finally, restoring landscapes to imagined pre-Columbian states is impractical. Del Tredici comments that:

> What I find particularly depressing about the 'native species only' argument is that it ends up denying the inevitability of ecological change. Its underlying assumption is that the plant and animal communities that existed in North America before the Europeans arrived can and should be preserved. The fact that this pre-Columbian environment no longer exists—and cannot be recreated—does not seem to matter (2004 p. 2).

Indigenous people and their land management techniques have largely been replaced with developed lands, leaving fractured remnant habitats lacking connectivity. Both law and risk regulate and reduce necessary disturbance regimes, such as fire, and many ecosystems have been undergoing either a drastically altered or complete lack of disturbance for centuries. Fencing, hunting, and fragmentation have greatly reduced or removed keystone animal species, such as large predators. Invasive plants, human disturbance, pollution, and chemicals have altered soils and microbial communities in many places. A changing climate will make pre-Columbian restoration even more difficult.

Herbicide dependency

Herbicide has become one of the major tools currently used by restorationists in the United States to help leap these hurdles. This is despite the fact that most herbicides were developed for agriculture, and have not been thoroughly tested in the context of ecological restoration. Many are known to or suspected of having detrimental effects on

human health, wildlife, native plants, and soil microbial communities (Wagner & Nelson 2014, Zaller et al. 2014). Katz would argue that using technology such as chemical herbicides to restore systems is inherently problematic ethically. It is also awkward for a discipline that espouses to be science-based to depend on practices where insufficient evidence on long-term effects exists. In fact, evidence showing that even herbicides such as glyphosate, the most widely used pesticide worldwide and considered one of the most environmentally friendly can have serious effects on rhizosphere keystone organisms such as earthworms and arbuscular mycorrhizal fungi, does exist. Recently (Zaller et al. 2014) found that glyphosate significantly reduced root mycorrhization and the biomass of AMF spores in soil. These detrimental effects occur both through the direct pathway of herbicide coming into contact with soil, and the indirect pathway of roots via foliage (Druille et al. 2013).

Restorationists continue to depend heavily on pesticide application despite scientific evidence and uncertainty because herbicide is one of the few available tools that allows for more than the smallest of areas to be treated. As humans we generally depend on visual metrics, and herbicide can provide what we want to see. Unfortunately many of the side effects, such as soil abiotic changes, microorganism decline, and even carcinogenic effects are invisible without costly and time-consuming studies and lab work. Depending on herbicide use without sufficient scientifically based evidence of the chemicals' long-term effects may undermine the credibility of restoration ecology within the scientific community.

An understanding of the harmful effects of pesticides has been part of popular knowledge since the publication of Rachel Carson's (1962) *Silent Spring* and the

emergence of serious medical problems in Vietnam veterans exposed to Agent Orange. Support of organic agriculture and skepticism of genetically modified crops and agro-chemical companies such as Monsanto, which manufactures glyphosate, have successfully spread from the environmental fringe to mainstream culture. Restorationists are likely alienating potential supporters by relying on herbicide use. Indigenous people may be especially reluctant to support or be involved in restoration projects that use herbicides, as the practice was introduced by Western culture, has no real counterpart in traditional management techniques, and could contaminate food and water resources. This conjecture is based only on hearsay, and research on the opinions of indigenous people regarding herbicide use in restoration is needed.

NOVEL ECOSYSTEMS

The concept of "novel ecosystems," which are defined as "[Ecosystems with] species compositions and relative abundances that have not occurred previously within a given biome . . . [and] result from biotic response to human-induced abiotic . . . or biotic elements . . . but do not depend on continued human intervention for their maintenance (Hobbs et al. 2006 p. 2)." Novel ecosystems can arise either from the degradation of more-natural ecosystems through human action or plant invasion, or the abandonment of heavily managed systems, such as agricultural fields (Hobbs et al. 2006). Humans have created novel ecosystems for millenia, but the number and spatial extent of these systems has increased rapidly in the modern era. Hobbs et al. (2006) argue that in this historical context, and because we cannot put Del Tredici's (2004) "invasive species genie back in the bottle," it makes sense to focus value questions on the ability of novel ecosystems to

provide services of equal value to the ones they are replacing. They argue simultaneously for:

> (1) conserving less impacted places now so they do not change into some new, possibly less desirable, form; and (2) not wasting precious resources on what may be a hopeless quest to 'fix' those systems for which there is little chance of recovery back to some pre-existing condition. Rather, we should perhaps accept them for what they are and what benefits they provide (Hobbs et al. 2004 p. 5).

Elliot and Katz would likely approve of this approach. It supports conservation and does not allow for malicious restoration. The novel ecosystems theory also leaves room for change and evolution, and does not attempt to "fix" nature through domination. These novel ecosystems can instead be seen as a part of humanity, and their current prevalence as reflective of a culture that values growth and the free hand of the market over conservation. Instead of denying what we are and what comes with that, this approach instead asks how we can work with what we have, plan for uncertainty, and make decisions that will allow novel ecosystems to provide necessary services.

CONCLUSION

Finding solutions to environmental issues, unfortunately, is usually more complex than it first appears. Even approaches that may initially seem inarguably positive, such as ecological restoration, are riddled with problems when scrutinized more closely. It is important, therefore, to know what problematics of logic or ethics commonly come up, and to develop a set of lenses through which to view and assess proposed environmental philosophies and methodologies. These should include, but not be limited to, the following:

1. Is a distinct line drawn between what is human and what is natural?
2. Are indigenous people and their history considered? Involved?

3. Are the needs of humans, and especially working-class people, considered? Involved?
4. Is long-term human domination required?
5. Is there the possibility of causing harm?
6. Are goals realistic and obtainable?
7. Do goals require stasis, or allow for change? Is climate change addressed?

I propose this as a framework for working within our current economic and social system, but acknowledge that real change to the relationships between humans, nature, and capital will likely be necessary to create a truly sustainable future. In my lifetime human-induced climate change has been exposed, gained nearly complete acceptance in the scientific community, and more recently among those with power in the world's governments. However, even with the looming presence of catastrophic warming that will have devastating effects on every aspect of human life, governments have proven incapable of agreeing upon or implementing solutions that could drastically slow or halt climate change (IPCC 2014). Earth has undergone at least five great extinction events prior to the current anthropogenic one, and life has recovered (Barnosky et al. 2011). Over geologic time scales, weathering and burial will return CO_2 to pre-industrial levels (Archer 2010). However, it is unknown whether our species will be able to effectively survive the chaos caused by a warmer climate and other anthropogenic environmental degradation.

Restoration ecology is a well-intentioned field that has developed some useful techniques and philosophies, and continues to evolve to address the concerns of its critics. Yet I do not agree with E. O. Wilson that it will be the means to end the great extinction

spasm, nor do I hope that the next century will be the "era of restoration in ecology" unless the discipline's current ethics and praxis undergo significant change. The good news is that even as Earth's population continues to grow and new ecological problems develop, environmental science and thinking are developing rapidly. New ideas are being constantly generated and tested, leading to an increase in knowledge that will affect and improve future practice. Restoration is a major force driving the understanding of both the potential and limits of applied ecology, and while still young and learning from mistakes, knowledge gained from today's experimentation will likely be a part of solutions in the future.

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