DISSERTATION

CHEATGRASS (*BROMUS TECTORUM* L.) INTERACTIONS WITH ARBUSCULAR MYCORRHIZAL FUNGI IN THE NORTH AMERICAN STEPPE: PREVALENCE AND DIVERSITY OF ASSOCIATIONS, AND DIVERGENCE FROM NATIVE VEGETATION

Submitted by

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ABSTRACT

CHEATGRASS (*BROMUS TECTORUM* L.) INTERACTIONS WITH ARBUSCULAR MYCORRHIZAL FUNGI IN THE NORTH AMERICAN STEPPE: PREVALENCE AND DIVERSITY OF ASSOCIATIONS, AND DIVERGENCE FROM NATIVE VEGETATION

Cheatgrass (*Bromus tectorum* L.) is a highly invasive winter annual grass that has caused significant changes to the steppe ecosystem of western North America. Cheatgrass is considered a facultative host of arbuscular mycorrhizal fungi (AMF), and has been shown to reduce AMF density in invaded soils and reduce AMF diversity in roots of neighboring grasses. However, specific information about interactions between cheatgrass and AMF remains unknown, as well as how these interactions differ from native vegetation. The research presented here addresses these knowledge gaps.

To determine when cheatgrass is colonized by AMF and the magnitude of colonization, two dense cheatgrass patches were identified in invaded shortgrass prairie in Colorado. Individuals were excavated every three weeks, from six weeks after germination through senescence. Roots were collected from individuals, cleared, stained, and observed for AMF colonization. Roots were colonized by AMF at every sampling date, but percent colonization of roots declined dramatically when soil temperatures

dropped below 0° C, and colonization remained low from late January through March. Peak colonization occurred in May (15.3%), when florets appeared on the cheatgrass shoots, and colonization dropped in June, once seeds were produced and senescence began. Although mycotrophic, cheatgrass is a poor host for AMF throughout its life, as evidenced by low AMF root colonization. Severe, lasting invasions by cheatgrass could have a negative impact on the AMF community.

Cheatgrass invasion is most severe in the sagebrush steppe of western North America, which is dominated by the native shrub big sagebrush (*Artemisia tridentata* Nutt.). As cheatgrass replaces big sagebrush, it is important to know how this shift affects the AMF community. Two studies were conducted to identify and compare AMF species associating with these two host plant species. Three sites (in Colorado, Utah and Wyoming) were selected where coexisting cheatgrass and big sagebrush populations were interspersed. Soil and root samples from underneath sixteen individuals of each species were collected at each of the sites.

In the first study, in which AMF species associating with big sagebrush and cheatgrass were identified, soil and root material was seeded with Sudangrass (*Sorghum bicolor* (L.) Moench ssp. *drummondii* (Nees ex Steud.) de Wet & Harlan), a promiscuous AMF host, and grown in the greenhouse for three consecutive culturing cycles. A total of 32 AMF species were identified from the trap cultures. Alpha diversity of AMF associated with big sagebrush was higher across all study sites compared to AMF associated with cheatgrass, although differences were only statistically significant across all sites. Gamma diversity was similar and beta diversity was higher in AMF associated with cheatgrass compared to big sagebrush. These results indicate that big sagebrush

individuals associate with more AMF species than cheatgrass and the sagebrush-associated AMF communities are more similar from one individual host to the next when compared to cheatgrass. Indicator species analysis identified two AMF species (*Archaeospora trappei* and *Glomus viscosum*) that were significantly more frequent in association with big sagebrush than cheatgrass across multiple sites. Identification of specific changes to the AMF community due to invasion, as suggested here, could lead to improved understanding of key plant-AMF interactions necessary for native plant recovery and restoration.

In the second study, AMF DNA was isolated from root and soil subsamples from source material for each of the trap cultures. A total of 27 unique AMF sequences were isolated from roots and soils. Although AMF diversity did not differ between host plants, AMF community composition in roots was significantly influenced by host, most likely due to half of the sixteen AMF species isolated from roots colonizing only one of the host species. This finding has important implications for invasion success and restoration of invaded soils, as alterations to the AMF community could provide positive feedbacks on the invader and decrease successful establishment of native plant species dependent on AMF.

In the final study, the interactions between early- and mid-seral native plant species and an AMF community associated with cheatgrass invasion were investigated. Plant responsiveness was measured using field soil with and without AMF. Soils used in the plant responsiveness study that contained AMF were collected and grown with a bioassay plant. AMF density was then measured by observing AMF colonization of the bioassay roots in the trained soils. Plant species studied were highly variable in their

interactions with AMF, and mutualisms, parasitisms, amensalisms and commensalisms were all observed. The presence of certain AMF facilitators may have a strong founder effect on plant communities and, where such feedbacks exist, identifying and utilizing these key interactions might facilitate the restoration of degraded ecosystems.

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DEDICATION

This dissertation is dedicated to my grandparents, Carolyn and Donald Higgins, who were taken from me not long before they had the chance to call their oldest grandson "Doctor". Their love of the outdoors was instilled in me from a very young age. Grandma shared her love of gardening with me, made the best homemade blackberry jelly, and appreciated my interest in preserving native plant species. Grandpa was my favorite mushroom hunting buddy, and many of my earliest memories of fishing, hunting, and four letter words include Grandpa. We shared so many interests for my entire life, and I owe a large part of my identity to them.

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Introduction

Plant invasions are a widespread and destructive consequence of human alteration of the environment (Vitousek et al. 1997). Numerous hypotheses have been generated to explain how plant invasions succeed, with the majority focused on interactions with other organisms (Mitchell et al. 2006). Among these hypotheses, interactions with the soil microbial community have been implicated in the successful invasion of numerous non-native invasive (hereafter "invasive") plant species (Richardson et al. 2000; Callaway et al. 2004; Wolfe and Klironomos 2005; Reinhart and Callaway 2006; van der Putten et al. 2007). Release of invasive plants from soil pathogens has been shown for some species (Klironomos 2002; van der Putten et al. 2007). The invasive plant Siamweed (*Chromolaena odorata* (L.) King & H. Rob.) can increase soil pathogens that strengthen the negative feedback on native neighbors (Mangla et al. 2008). Alterations to other aspects of the soil microbial community by invasive plants have also been confirmed, including changes to microbial community structure and rates of decomposition (Holly et al. 2009), fungal:bacterial ratios (Niu et al. 2007), *Acidobacterium* (Kuske et al. 2002), sulfate reducing and sulfur oxidizing bacteria (Batten et al. 2006), and ammonia-oxidizing bacteria (Hawkes et al. 2005). There is also growing evidence that soil

mutualisms, particularly arbuscular mycorrhizal fungi (AMF), can be important in the successful invasion of introduced plants (Richardson et al. 2000; Reinhart and Callaway 2006; van der Putten et al. 2007; Shah et al. 2009; Pringle et al. 2009).

AMF have a global distribution, have little specificity for host colonization and associate with many terrestrial plant species (Smith and Read 2002). Because of their widespread occurrence and direct association with many plant species, AMF can be highly influential on host plants. Many plant species can be colonized by AMF (often multiple AMF simultaneously), and AMF can simultaneously colonize multiple host plants (Read 1998). Through this complexity, plants and AMF can derive benefits. However, the benefits are not assured. AMF are not strictly mutualists, but can range from mutualistic to parasitic on plant hosts (Francis and Read 1995; Johnson et al. 1997; Jones and Smith 2004). However, much more information is known about plant responses than fungal responses, and it is generally assumed that AMF always derive benefits from colonized hosts.

Phosphorus nutrition is the primary benefit provided to plants by AMF, although AMF also increase uptake of Zn, Cu, and N, and improve water relations of hosts (Smith and Read 2002). Subsequently, plant response to AMF colonization is most positive when soil is P-limited (Hoeksema et al. 2010). Plant functional group identity and N-status of the soil are other important factors influencing plant responses to AMF (Hoeksema et al. 2010). Plant taxonomic grouping can be a strong indicator of interaction strength, as non-mycorrhizal plant species are concentrated in specific plant families (Pendleton and Smith 1983; Newman and Reddell 1987; Wang and Qiu 2006). Non-mycorrhizal plant species grow best in the absence of AMF (Francis and Read

1995), obligate mycorrhizal plant species grow best in the presence of AMF (Hartnett et al. 1993; Hartnett and Wilson 1999), and facultative mycorrhizal plant species generally show little preference (Hartnett et al. 1993; Hartnett and Wilson 1999). Perennial plant species are normally more responsive to AMF than annual plant species (Boerner 1992a). Plant species most responsive to AMF are C_4 grasses, woody plants and non-N-fixing forbs, while plant species least responsive to AMF include forbs associating with N-fixing symbionts and C_3 grasses (Hetrick et al. 1988; 1990; Wilson and Hartnett 1997, 1998; Hoeksema et al. 2010). Forbs are highly variable in response to AMF (Wilson and Hartnett 1998), but perennial forb establishment is much higher in the presence of AMF (Gange et al. 1993). Forb species with greater root fibrousness tend to be more dependent on AMF (Hetrick et al. 1992).

Different AMF species provide different benefits to their hosts, and different combinations of AMF species in a community provide different host growth responses (Gustafson and Casper 2006). The host benefits of simultaneous AMF colonization are dependent on the identities of both host plant and AMF species (Jansa et al. 2007). However, colonization by multiple AMF species has also been shown to provide no identified additional benefits over colonization by individual AMF species (Janoušková et al. 2009), and colonization of a single plant host by multiple AMF has even been shown to reduce host benefit compared to the individual AMF species (Violi et al. 2007). Thus, colonization by multiple AMF species simultaneously alters host benefit, and appears to be dependent on host and AMF species identity.

Existing vegetation colonized by AMF can improve establishment of conspecific seedlings through shared AMF networks, and the effects are dependent on both plant and

AMF species (van der Heijden 2004). Although seedlings grown with mycorrhizal adult plants are more highly colonized by AMF, the effects of competition with large neighbors can negate any benefits provided by the AMF networks (Eissenstat and Newman 1990; Kytöviita et al. 2003). Thus, the role of AMF in plant recruitment also appears to vary by both host and AMF identity.

Plant community composition is also influenced by AMF (Janos 1980). AMF reduce the effects of plant species loss on plant community productivity (Klironomos et al. 2000). Different AMF species can preferentially colonize different host plant species, and when colonizing multiple hosts can provide different benefits to the different hosts (Helgason et al. 2002; Castelli and Casper 2003). Higher diversity of AMF has been shown to promote higher productivity and diversity of plant communities, but AMF species identity is important (van der Heijden et al. 1998a; van der Heijden et al. 1998b; Vogelsang et al. 2006). Vogelsang et al. (2006) further found that identity of AMF species was more important than total number of species. Apparently, quality of AMF species is more important than quantity when considering plant community diversity and productivity. This finding was further supported by Oliveira et al. (2006).

Klein et al. (2006) have suggested that a mycocentric approach to management of the soil community could improve our understanding of mechanisms of interactions between invasive and native plant species. While it has been shown that AMF can play a role in successful plant invasions, a basic understanding of the importance of AMF is lacking for most plant invaders and environments. Utilizing AMF to combat plant invasion will require thorough understanding of the complexity of plant-AMF interactions. Such information is currently lacking for application to any specific

occurrence of invasion. The following review will highlight this complexity, providing an overview of what is known about plant-AMF interactions and plant invasion, and focus on how this knowledge can be expanded for use in restoring invaded systems.

The Role of AMF in Plant Invasion

Combining the effects of AMF diversity on plant diversity and plant diversity on AMF diversity is the Driver/Passenger Hypothesis (Hart et al. 2001). Under this hypothesis, certain AMF species are responsible for plant community changes while other AMF species are the product of plant community changes (Hart et al. 2001). This reasoning has also been applied to understanding the role of AMF in plant invasions (Shah et al. 2009). AMF can drive plant invasion if they establish relationships that favor mycorrhizal plant invaders, or they can be passengers of plant invasion if the invasion alters AMF communities in a way that reduces the benefits provided to native plants (Figure 1).

Non-Mycorrhizal and Non-Responsive Invaders

Many invasive plant species are non-mycorrhizal (Pringle et al. 2009). However, many of the most widespread and aggressive invaders appear to be associated with AMF (Table 1). Unfortunately, information on the AMF associations for numerous plant invaders is lacking (Pringle et al. 2009; Shah et al. 2009). Plant invasions by non-mycorrhizal species can cause major shifts in the mycorrhizal status of dominant plant species. Garlic mustard (*Alliaria petiolata* (M. Bieb.) Cavara & Grande), a non-mycorrhizal plant invader, suppresses AMF in invaded soils through exudates (Roberts and Anderson 2001; Stinson et al. 2006). Surprisingly, this suppression is much stronger

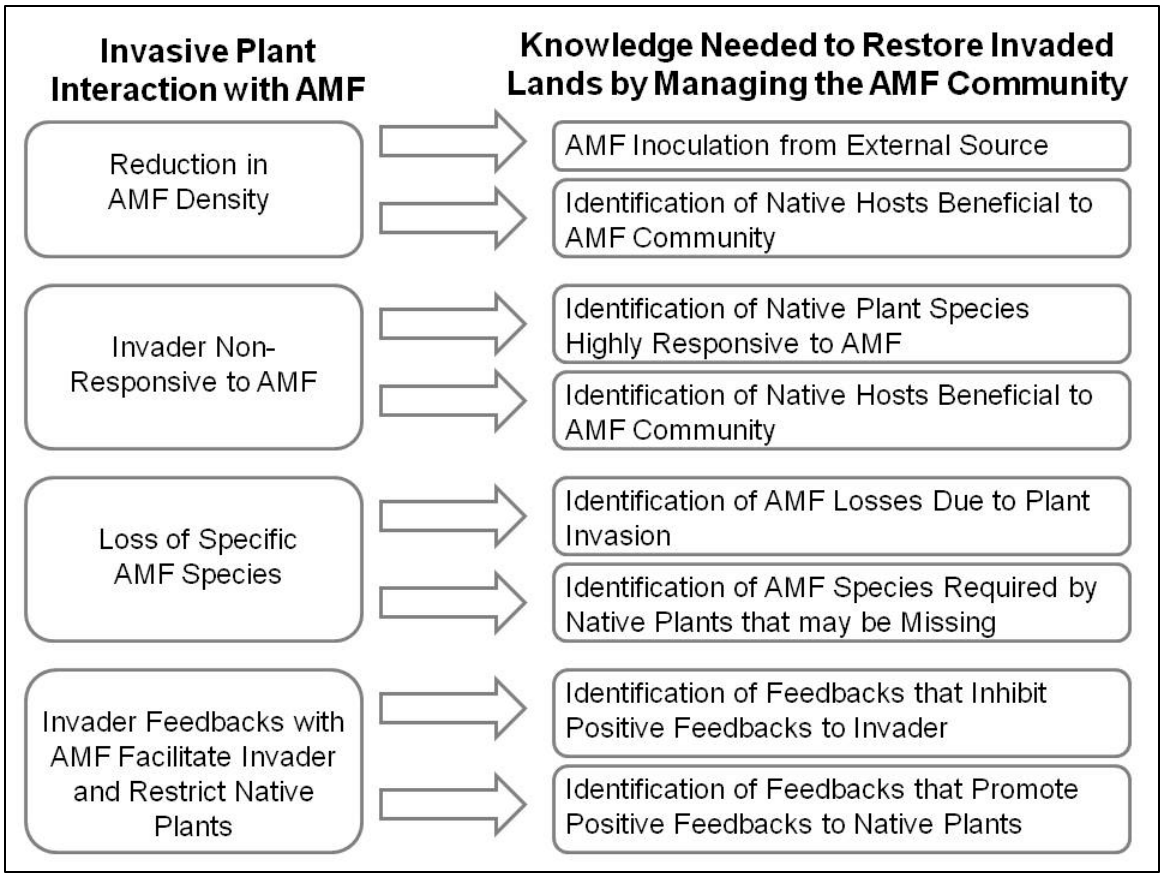

Figure 1. Knowledge needed to affect the AMF community given specific interactions between plant invaders and the AMF community.

on AMF in invaded soils than in its native range, indicating AMF adaptation to this exudate in garlic mustard's native range (Callaway et al. 2008). Russian thistle (*Salsola kali* L.) can become colonized by AMF, but necrotizes colonized root cells to its own initial detriment (Allen et al. 1989). In disturbed soils, persistence of non-mycorrhizal plant species such as Russian thistle can reduce AMF density (Christensen and Williams 1977).

Invasive mycorrhizal plants may not be as dependent on AMF in their introduced ranges relative to their native ranges (Seifert et al. 2009). Some introduced plant

Table 1. Mycorrhizal status of twenty five widespread and aggressive plant invaders.

Invasive Plant	Vegetation Type	Native Range[1]	Invasive Range[1]	Mycorrhizal Status[2]
Acacia mearnsii	perennial shrub/tree	Au[3]	Af, As, Eu[3]	Unknown
Ageratum conyzoides	annual/biennial forb	NA, SA	Af, As, Au, NA, SA, is	AM[4]
Alliaria petiolata	annual/biennial fob	Af, As, Eu	NA, is	NM[4]
Bromus rubens	annual grass	Af, As, Eu	Au, NA	AM[4]
Bromus tectorum	annual grass	As, Eu	Au, NA	AM[4]
Buddleja davidii	perennial shrub	As	Au, Eu, NA, is	NM[4]
Centaurea stoebe (= *biebersteinii*[1])	biennial/perennial forb	Eu	NA, SA	AM[4]
Chromolaena odorata	perennial subshrub	NA, SA	Af, As, Au, NA, is	AM[4]
Impatiens glandulifera	annual forb	As	Eu, NA	AM-NM[4]
Imperata cylindrica	perennial grass	As	Af, As, Au, Eu, NA, SA, is	AM[4]
Lantana camara	perennial shrub/vine	NA, SA	Af, As, Au, NA, is	AM[4]
Leucaena leucocephala	perennial shrub/tree	NA, SA	Af, As, Au, NA, SA, is	AM[4]
Miconia calvescens	perennial shrub/tree	SA	Af, As, Au, Eu, is	Unknown
Mimosa pigra	perennial shrub	NA, SA	Af, As, Au, NA, is	Unknown
Parthenium hysterophorus	annual forb	NA, SA	Af, As, Au, NA	AM[4]
Pennisetum ciliare (= *Cenchrus ciliaris*[1,4])	perennial grass	Af, As, Eu	Au, NA, SA	AM[4]
Polygonum cuspidatum	perennial forb/subshrub	As	Au, Eu, NA, is	AM-NM[4]
Prosopis glandulosa	perennial shrub/tree	NA	Af, As, Au	AM[4]
Salsola kali (= *tragus*[1])	annual forb	Af, As, Eu	Au, NA	AM[4]
Solidago canadensis	perennial forb	NA	As, Au, Eu, is	AM[4]
Sorghum halepense	perennial grass	Af, As, Eu	As, Au, NA, is	AM[4]
Tamarix aphylla	perennial shrub/tree	Af, As	Au, NA	AM[5]
Tamarix ramosissima	perennial shrub/tree	As	Af, Au, NA, SA	AM-NM[6]
Ulex europaeus	perennial shrub	Eu	As, Au, NA, SA, is	AM[4]
Urochloa maxima (= *Panicum maximum*[4])	perennial grass	Af	As, Au, NA, SA, is	AM[4]

[1]From ISSG (2008), except *Solidago canadensis*: Weber (2000)

[2]AM = arbuscular mycorrhizal, NM = non-mycorrhizal, AM-NM = AM and/or NM (contrasting results in different studies)

Table 1 (continued).

[3]Af = Africa, As = Asia, Au = Australia, Eu = Europe, NA = North America, SA = South America, is = islands
[4]As reviewed by Wang and Qiu (2006)
[5]Mathur and Vyas (2000)
[6]Beauchamp et al. (2007)

communities can reduce AMF densities compared to native plant communities, and subsequent growth by native vegetation is diminished in the invaded soil (Vogelsang and Bever 2009). This phenomenon has been described as the degraded mutualist hypothesis (Vogelsang and Bever 2009). Cheatgrass (*Bromus tectorum* L.*)*, an invasive annual grass, supports this hypothesis through its interactions with AMF in invaded soils. Cheatgrass is a facultative host of AMF and shows no growth response with or without AMF (Allen 1984b). Additionally, soils from cheatgrass-dominated areas may have lower mycorrhizal inoculum than uninvaded soils (Al-Qawari 2003).

These alterations can suppress establishment of native plant species and support the dominance and persistence of the invaders (Vogelsang and Bever 2009). This adaptation could allow invasive plants to affect reductions in AMF density themselves, or exploit circumstances where prior disturbances have reduced the AMF community. Soil disturbance can reduce AMF density and species diversity (Helgason et al. 1998; Oehl et al. 2003). Such a reduction in AMF could favor plant species less dependent on AMF. Where AMF are reduced in soils, fewer plant species may establish (Gange et al. 1990).

Highly Responsive Mycorrhizal Invaders

AMF could facilitate plant invasion simply by providing a greater benefit to invasive host plants than to native plants. Variable responses to AMF between native and invasive host plants have been shown (Wilson and Hartnett 1998; Zhang et al. 2010). Comparisons between tallgrass prairie plants in North America using a mixed AMF inoculum indicated that an invasive C_4 perennial grass, Caucasian bluestem (*Andropogon bladhi* (Retz.) S.T. Blake), was more responsive to AMF than 10 of 13 native C_4

perennial grass species (Wilson and Hartnett 1998). The invasive C_3 perennial grass, tall fescue (*Festuca arundinacea* Schreb.), was more responsive to AMF than the 6 native C_3 perennial grass species studied. The invasive legume, sericea lespedeza (*Lespedeza cuneata* (Dum. Cours.) G. Don), was more responsive to AMF than 12 of the 14 native legumes studied. The invasive biennial forb, bull thistle (*Cirsium vulgare* (Savi) Ten.), was by far the most responsive of the 12 annual and biennial forbs studied. Thus, mycorrhizal plant invaders can receive greater benefits from AMF compared to native plants.

Spotted knapweed has been shown to obtain a competitive advantage over the native grass, Idaho fescue (*Festuca idahoensis* Elmer), through C transfer by shared AMF, and by supporting greater hyphal growth that subsequently supplies more P to spotted knapweed than native grasses (Marler et al. 1999; Zabinski et al. 2002; Carey et al. 2004; Walling and Zabinski 2004). However, the Marler et al. (1999) study used a mixed AMF inoculum of unknown composition and found greater spotted knapweed growth responses with Idaho fescue than with another perennial native grass, blue grama (*Bouteloua gracilis*). It is plausible that differences in AMF species preferences by the native grasses allowed differential benefits to be obtained by the invader. AMF from early successional soils also provide greater benefits to spotted knapweed (*Centaurea stoebe* ssp. *micranthos* (Gugler) Hayek) than AMF from later successional soils (Harner et al. 2010). Like plants, AMF species vary in their occurrence with respect to time since disturbance, and some species are early successional while others are late successional (Johnson et al. 1991). It is possible that certain AMF species are favored by spotted knapweed, which may allow it to perform better in the early successional soils.

AMF Community Changes in Invaded Soils

Plant community composition has a strong influence on AMF community composition (Johnson et al. 1992; Scheublin et al. 2004). Because AMF species diversity may rival plant species diversity in communities (Öpik et al. 2009), loss of native plant diversity associated with plant invasions may result in AMF species losses as well. Mycorrhizal plant invaders can alter the AMF community, which may facilitate plant invasion (Stampe and Daehler 2003; Pringle et al. 2009).

Invasive plants can increase overall AMF abundance in invaded soils (Niu et al. 2007). Greipsson and DiTommaso (2006) found that AMF densities were higher in soils invaded by three invasive plants than in uninvaded areas. However, other invasive plants decrease AMF in invaded soils. Native grasses growing in the presence of cheatgrass have lower diversity of AMF colonization than when grown without cheatgrass (Hawkes et al. 2006). Spotted knapweed may also reduce AMF diversity (Mummey and Rillig 2006).

Some invasive plant species appear to alter AMF communities by showing preferences for certain AMF species. Indian sandbur (*Cenchrus biflorus* Roxb.) was shown to have a higher frequency of AMF colonization by *Glomus versiforme* than two coexisting native grasses (van der Putten et al. 2007). Chamomile (*Anthemis cotula* L.) and horseweed (*Conyza canadensis* (L.) Cronquist) associated with fewer AMF species than were present in nearby un-invaded sites, and spore density of the AMF species they associated with was higher in the invaded soil than in the nearby un-invaded soil (Shah et al. 2010). Goldenrod (*Solidago canadensis* L.) altered the AMF community by increasing one species of AMF (*Glomus geosporum*) while reducing another (*Glomus*

mosseae) (Zhang et al. 2010). Spotted knapweed also strongly influenced the AMF species colonizing roots of a neighboring grass (Mummey et al. 2005).

Unfortunately, species richness of AMF communities is difficult to determine (Rosendahl 2008). Even more difficult is distinguishing the effects of plant invasions on AMF from all other environmental factors. Individual host plant species have been shown to associate with different AMF species at different sites, indicating that factors other than host identity are important (Kennedy et al. 2002; Schechter and Bruns 2008). AMF species associating with *Festuca* species across 27 sites were influenced by site (Molina et al. 1978). AMF species associated with big sagebrush (*Artemisia tridentata* Nutt.) varied across 48 sites (Allen et al. 1995). Thus, AMF community composition at any given location is more a function of environmental conditions than host identity (Allen et al. 1995). Many environmental factors influence AMF communities, including soil disturbance (Moorman and Reeves 1979), soil acidity (Moutoglis and Widden 1996), herbivory (Klironomos et al. 2004; Bennett and Bever 2007), nutrient deposition (Johnson 1993; Sigüenza et al. 2006), and fire (Wicklow-Howard 1989). The absence of an AMF species may be due to numerous factors in addition to plant invasion.

Managing the AMF Community

Knowledge of factors influencing plant interactions with AMF, and the difficulties associated with studying these interactions have important potential implications for restoration of invaded soils (Figures 1 and 2). A mycocentric approach to restoring invaded soils requires knowledge of AMF species that may be lost due to invasion, as well as knowing what AMF species are favored by native plant species. AMF species are unique, and possess distinct colonization strategies (Hart and Reader

2002), tolerances to soil disturbance (Merryweather and Fitter 1998) and foraging strategies (Gavito and Olsson 2008). Several species of AMF have global distributions, while many others have been isolated from only one site (Öpik et al. 2006). Knowledge of whether or not invasion-driven AMF alterations are inhibiting native vegetation is also important for utilizing AMF to restore invaded lands. The establishment of a particular plant species may depend on the availability of certain AMF species (Renker et al. 2004). Thus, the loss of even one AMF species from a site might cause major alterations to the plant-soil system (Allen et al. 1995).

Increasing AMF in Invaded Soils

If plant invaders are non-mycorrhizal or able to alter AMF when mycorrhizal, simply adding AMF to an invaded soil may improve recovery of native vegetation. Stimulating AMF has been suggested as important for controlling non-mycorrhizal weedy plant species (Jordan et al. 2000; Cameron 2010; Rinaudo et al. 2010). One approach to combat plant invasion is the use of AMF soil inoculants, either from commercial sources or from an appropriate reference community. Inocula can be added directly to a site or inoculated containerized stock can be transplanted. Inoculating a site with foreign AMF can lead to positive or negative growth responses in hosts (Onguene and Kuyper 2005). Introducing AMF into new sites causes changes in spore composition, can alter plant responses, and changes plant interactions (Yao et al. 2008; Ji et al. 2010). AMF species used as host pre-inoculants can restrict subsequent colonization by indigenous AMF species, and alter responses of host plants (Mummey et al. 2009). Plant species also respond differently to exotic AMF than to native AMF, and vice versa (Klironomos 2003). AMF inoculants can also be ineffective (Rowe et al.

2007). Propagation of AMF species under controlled settings can select for certain traits that may not be advantageous under field settings (J. Morton, personal communication). Further, host plant species identity is associated with genetic diversity changes in AMF species (Ehinger et al. 2009). Thus, the act of producing the inoculum may reduce its usefulness.

Local whole-soil inoculum from a desirable native plant community can improve native plant restoration (Smith et al. 1998; Rowe et al. 2008). Native AMF may be better for shrub establishment than introduced AMF (Caravaca et al. 2003). However, mismatches in soil microbial community type and target vegetation may cause soil inoculation to be ineffective (Kardol et al. 2009). Tree seedlings may grow better with early-successional inocula compared to late-successional inocula, regardless of host growth strategy (Allen et al. 2003). However, AMF taken from the same native host plant species may not persist when transplanted to another site with the same host (Weinbaum et al. 1996).

In areas where AMF is abundant or where disturbance has not drastically reduced local AMF populations, inoculation may not be necessary (White et al. 2008). In arid and semi-arid systems, shrubs persisting at disturbed sites can serve as AMF resource islands that support higher densities of AMF than the shrub interspaces (Azcón-Aguilar et al. 2003; Camargo-Ricalde and Dhillion et al. 2003). The same may be true if patches of other types of remnant vegetation exist at an invaded site.

Utilization of Highly Responsive Native Plants

Although many factors can negatively affect AMF, their diversity in soils can remain high even with a history of severe and repeated soil disturbance and fertilization

(Hijri et al. 2006). AMF can be present in fields with a long history of intensive agriculture (Franke-Snyder et al. 2001), and AMF communities below the depth of severe soil disturbance can be more diverse and different from the topsoil AMF community (Oehl et al. 2005). AMF spores are mobile, and can naturally re-colonize disturbed sites (Allen 1987; Warner et al. 1987). Thus, even in soils where soil disturbance is severe and common, it is very difficult to completely remove AMF.

However, while AMF diversity may withstand severe disturbances, AMF density can be significantly reduced in soils following disturbance (Moorman and Reeves 1979). Because of this loss of AMF, plant colonizers are often either non-mycorrhizal or facultative hosts (Miller and Jastrow 1992). Plant community assembly processes appear to be closely tied to the AMF community, as higher densities of AMF support higher rates of plant succession following disturbance (Allen and Allen 1984; Doerr et al. 1984; Allen 1988). Plant community assembly after disturbance often follows a general pattern of colonization by less mycotrophic plant species to later stages composed of highly mycotrophic plant species (Allen 1984b). Plant species persisting longest during succession are the most responsive to AMF (Boerner 1992b) and dependence of the plant community on AMF increases with plant community age (Allen 1984a). Thus, improving AMF density could significantly improve establishment of mycorrhizal native plant species.

Generally, a small number of native species from a large species pool are repeatedly utilized for ecological restoration. However, species possessing diverse functional traits are recommended for restoring invaded soils (Funk et al. 2008). Diversity of plant functional groups improves community resistance to invasion (Pokorny

et al. 2005). Because AMF specificity also appears to occur at the functional group level (Öpik et al. 2009), reestablishment of diverse host groups may reintroduce hosts that are best adapted to benefit from the AMF community at a site. However, restoring invaded soils may be difficult if native species utilized are not able to benefit from the altered AMF community.

Many weedy native species overlooked for restoration may be adapted to this type of environment, and could prove useful for improving conditions for more desirable, AMF-dependent native plant species. Many non-mycorrhizal plants are also annuals, but not all annuals are non-mycorrhizal (Wang and Qiu 2006). Many of these annual pioneer species are negatively responsive to AMF, but others can be non-responsive or even positively responsive (Vatovec et al. 2005; Rinaudo et al. 2010). Some native plant species appear to be resistant or insensitive to invasive plant alterations to the soil microbial community (Jordan et al. 2008). Thus, pioneer species may be more complex in their interactions with AMF than commonly believed (Pezzani et al. 2006). The most efficient approach to identify native plant species highly responsive to an AMF community is to compare AMF responsiveness of potential hosts to the plant invader(s) under controlled settings. However, AMF benefits to a plant host depend not only on AMF and plant species, but also local environmental conditions (Reynolds et al. 2006). So, it is important to know responses of plant species specific to the restoration site. Then, native plant species exhibiting high positive responses to a specific AMF community could be selected for restoration activities at that site. Assessing a large number of potential native species will provide the greatest likelihood of identifying plants that are more responsive than the invaders.

Utilization of Native Plants Most Beneficial to AMF

Another alternative is the selection of native host species providing the most favorable benefits to the AMF community in an invaded soil. Responses of AMF to hosts may be more important ecologically than responses of hosts to AMF. This is due to the legacy effects of host plant species on the AMF community and the ability of AMF to associate with different hosts simultaneously. Unfortunately, the mycorrhizal status of numerous plant species remains unknown. Further, the effects of most mycorrhizal plant species on AMF have not been studied. It is likely that certain native species interact more favorably with AMF than others, and these species may possess the ability to rapidly increase AMF in soils. These hosts themselves may not be the most responsive to AMF, but instead provide the greatest benefits to AMF. Such changes to the AMF community could improve site conditions for other native plants that are more dependent on AMF.

AMF specificity may occur at host functional group level rather than host plant species level (Öpik et al. 2009). However, AMF host preferences have been identified between coexisting plant species (Vandenkoornhuyse et al. 2002), and even between functionally similar perennial C_3 grasses (Vandenkoornhuyse et al. 2003) and perennial C_4 grasses (Castelli and Casper 2003). Aside from AMF specificity for hosts, many other factors influence colonization of plant species. Identity of plant neighbors influences AMF colonization (Jastrow and Miller 1993) and species composition (Hausmann and Hawkes 2009) of host roots. The AMF species colonizing forest understory plants are influenced by the composition of the overstory (Helgason et al. 1999). Priority effects of plant host establishment may influence AMF communities in subsequent host plants

(Hausmann and Hawkes 2010). Growth stage of the host also affects benefits derived by associations with AMF. AMF species associating with seedlings change as the host species age (Husband et al. 2002). Plant species also vary in responses to different AMF at different life stages, across seasons and across years (van der Heijden et al. 2006). Thus, what works well at a given site may not work the following year, or perceived desirable interactions during establishment may be undesirable as plants age.

Ecological genetics in plant restoration is another important consideration for a variety of reasons (McKay et al. 2005), and the potential for problems with genetic mismatches in plant-AMF interactions is no different. AMF-host plant interactions can differ depending on conspecific host plant genotypes (Ronsheim and Anderson 2001; Pánková et al. 2008), and AMF colonization varies widely by host plant genotype (An et al. 2010). On the other hand, certain AMF genotypes within an AMF species exhibit host plant species preferences (Croll et al. 2008), and genotypes of individual AMF species differentially affect growth in host plants (Koch et al. 2006). Host plant species identity can be associated with genetic diversity changes in AMF species (Ehinger et al. 2009), and genetically diverse plant communities associate with fewer AMF species than genetically deficient plant communities (Johnson et al. 2010). Given the importance between interactions and genotype, consideration must also be given to native seed sources and AMF compatibility when restoring invaded soils. It is possible that different seed lots of the same species could interact in desirable and undesirable ways with the same AMF community if the genetic diversity is dissimilar (Schultz et al. 2001; Cavender and Knee 2006). If so, restoration success might depend entirely on the source of seeds

used. Thus, plant-AMF specificity is a product of numerous factors that must be considered when utilizing AMF for restoration.

Controlling Feedbacks through Alternative Hosts

Indiscriminate promotion of all AMF species resulting from plant invasion through selection of beneficial hosts may not create conditions favorable for restoration of desirable native plants. Some of the existing AMF species in an invaded soil may have facilitated the invasion to begin with. Instead, utilization of feedbacks to explicitly control AMF species composition may be the most influential means of manipulating plant-AMF interactions in invaded soils (Figure 2).

Host plant species and host strategy have strong influences on AMF community composition (Johnson et al. 1992), while AMF species differ in their ability to successfully colonize host plants (Daniels et al. 1981). Further, plants can promote more beneficial AMF species by preferentially providing greater benefits to them (Bever et al. 2009). Understanding the specific responses between native and introduced plant species with associated AMF may yield particular interactions that can be exploited to restore invaded soils, as different AMF species alter competitive outcomes between plant species (Bever et al. 2002; van der Heijden et al. 2003; Oliveira et al. 2006; Scheublin et al. 2007).

AMF-host plant interaction specificity can produce feedbacks that either favor or inhibit certain species (Bever et al. 2002). Depending on the degree of these feedbacks, the associated species can be excluded, dominate or coexist in communities. One such example is presented by Bever (2002). The AMF species (*Acaulospra morrowiae* and

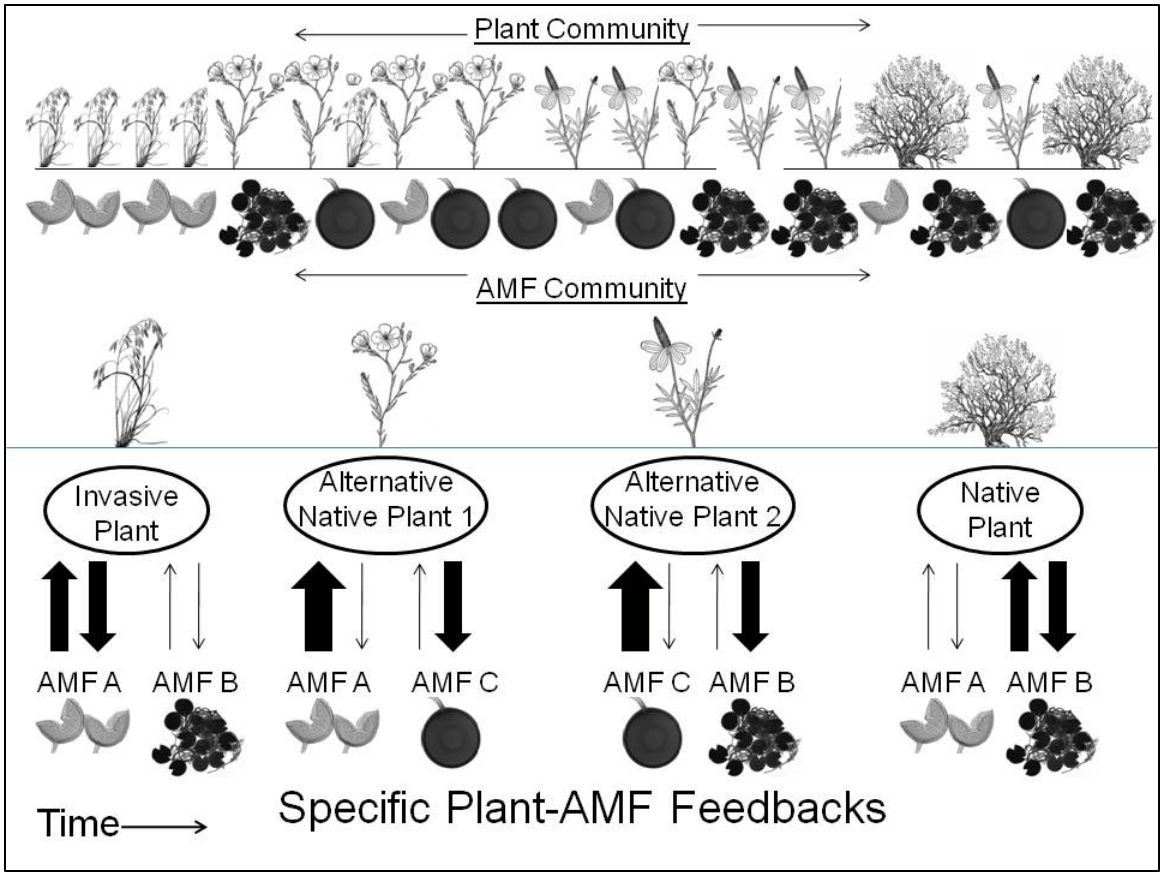

Figure 2 Conceptual diagram of a potential approach using native plant-AMF feedbacks to control invasive plant-AMF feedbacks. Plant and AMF community figures represent relative densities of species over time. Specific plant-AMF feedbacks adapted from Bever (2003). Widths of arrows represent relative strength of benefits provided. In this approach, the invasive plant favors AMF A, and AMF A favors the invasive plant. The invasive plant neither provides benefits for nor receives benefits from AMF B, which is favored by the desired native plant but low in density due to feedbacks resulting from invasion. This negative feedback on the native plant would restrict its establishment in the invaded soil. However, alternative native plant 1 receives greater benefits from AMF A than does the invasive plant, but provides greater benefits to AMF C than AMF A. Under these circumstances, growth of alternative native plant 1 in the invaded soil would result in a strong positive feedback on alternative native plant 1 and a strong positive feedback on AMF C. Alternative native plant 2 derives significant benefits from AMF C, but provides greater benefits to AMF B, increasing it in the soil. Alternative native plant 2 would then generate a positive feedback when grown in soil "trained" by alternative native plant 1 while increasing AMF B. The increase in AMF B then favors the desired native plant through beneficial association, and this association would generate a positive feedback on the native plant. Line drawings from left to right: cheatgrass (*Bromus tectorum* L.), Lewis flax (*Linum lewisii* Pursh), prairie coneflower (*Ratibida columnifer* (Nutt.) Woot. & Standl.) courtesy of United States Department of Agriculture, and big sagebrush (Artemisia tridentata Nutt.) courtesy of United States Department of the Interior. Photos of AMF, from left to right: *Glomus aggregatum* N.C. Schenck & G.S. Sm., *Glomus fasciculatum* Gerd. & Trappe (as fasciculatus), and *Glomus constrictum* Trappe (as constrictus) by R. Busby.

Archaeospora trappei) that 1 introduced host plant species (*Plantago lanceolata* L,) is most responsive to actually prefer a native grass (*Panicum sphaerocarpon* Elliot), while the AMF that prefers *Plantago* (*Scutellospora calospora*) is less beneficial than AMF that associate with the neighboring *Panicum* (Bever 2002). In this type of negative feedback, an increase in one host plant species provides a positive feedback for the neighboring plant species and a negative feedback on itself through differential associations with AMF.

It remains unknown if interactions between introduced and native plant species with AMF differ so drastically that the invader is able to exclude native plants through plant-AMF feedbacks, and vice versa. In other words, can other feedbacks thwart the feedbacks that favor the invader? Aside from the feedbacks presented by Bever (2002), few studies have detailed how different associations between native and introduced plant species even differ with specific AMF species. The best evidence comes from Zhang et al. (2010), where goldenrod increases one AMF species (*Glomus geosporum*) while reducing another (*Glomus mosseae*). This shift in AMF associations provides a positive feedback on goldenrod and a negative feedback on native Korean clover (*Kummerowia stipulacea* (Maxim.) Makino). However, if certain native plant species were identified that were poor hosts for *Glomus geosporum* and superior hosts for *Glomus mosseae*, promotion of these feedbacks might restrict invasion of the goldenrod. Consideration must also be given to native hosts that are superior hosts for AMF such as *Glomus geosporum* that are preferred by the invader and alternative native plant hosts that are most dependent on this AMF species. Native plant species that potentially produce positive feedbacks for invaders may require control themselves to avoid facilitating the

invader. Furthermore, native plant species that depend upon the same AMF species as invaders may be lost if controlling the invader requires suppressing these AMF feedbacks. The number of native species that both promote and depend on specific invasive plant-promoting AMF could be staggering.

The study by Zhang and coworkers (2010) comparing feedbacks with goldenrod and Korean clover is the best example to date of utilizing plant-AMF feedbacks to understand plant invasion. Unfortunately, detailed comparisons beyond this level do not exist. To utilize these feedbacks to restore invaded sites, knowledge well beyond what currently exists will be needed. Two plant-two AMF systems are much simpler than natural communities. Specific host plant species interactions with AMF are unknown for most plant species. At any given site, the potential vegetation composition resulting from the local plant community and from the ecosystem as a whole is tremendous, and many plant species have never been investigated. Many of these understudied species could possess unique interactions with AMF, and could hinder or facilitate AMF species important to either plant invaders or desirable native plant species (Figure 2).

Feedbacks through AMF undoubtedly occur between host plants from earlier seral states with native plant species that are more competitive. Nexus species are species that perform a necessary ecological function, but are not present when the importance of that function is realized (Lockwood and Samuels 2004). Thus, ruderal and other overlooked native species could possibly serve as nexus species by affecting the AMF community in a manner that is necessary for subsequent establishment by more competitive plant species. Because of the legacy effects of plant species on the AMF community (Hausmann and Hawkes 2010), those species that are ruderal may have strong legacy

effects on the AMF community encountered by more competitive native species. Assembly history can also influence the spatial variation in AMF community composition in a plant host population (van de Voorde et al. 2010). Different restored plant species may associate with different AMF species, indicating that restoring a greater diversity of host plant species may result in greater AMF diversity (Alguacil et al. 2011). Thus, the effects of understudied native plant species on the AMF community are likely important for establishment of more desirable native species. However, these feedbacks must be identified before being exploited.

Conclusions

Plant associations with AMF are highly variable, and the effects of these interactions depend on numerous factors. The outcomes of these associations have a strong influence on plant community composition. There is growing evidence to suggest that AMF interactions with host plants also play an important role in plant invasions. Non-mycorrhizal and mycorrhizal invaders with reduced dependence on AMF may be able to exploit disturbed soils that are missing a suitable AMF component, or reduce AMF that are needed by native plants. Other invaders may be able to exploit pre-existing AMF to exert dominance over native species. Identifying AMF species involved in invader success, missing from or reduced in invaded soils, and necessary for native species recovery would significantly improve our knowledge of the importance of AMF in plant invasion. However, understanding how to manipulate an AMF community is required in order to apply this knowledge for restoration of invaded sites.

Understanding which AMF species are involved in invader success may be unimportant for many invasive plants, as they are non-mycorrhizal. Others may not be

responsive to AMF and will readily associate with any available AMF species. However, some may be more selective and show preference for certain AMF species across an invaded range. For restoring areas invaded by non-mycorrhizal and non-responsive invaders, identifying native plant species that benefit most from AMF inhabitants may prove useful. Increasing AMF density in invaded soils may allow certain native species to reestablish. However, adding AMF to a site is potentially unfavorable due to unknown interactions with hosts. Alternatively, native plants could be identified and utilized that are efficient at promoting the indigenous AMF remaining after invasion.

Understanding which AMF species are missing from invaded soils requires considerable investigation. AMF community characterization is a difficult task, and AMF community changes due to plant invasion must account for additional impacts of environmental factors. AMF community differences are often site-specific, so determining localized AMF species extinctions due to a plant invasion may be extremely difficult. On the other hand, understanding which AMF species are necessary for native species recovery is simpler than identifying AMF species lost due to invasion. Remnant populations of native plant species should provide information necessary to determine which AMF are preferred by native plants, which are not, and which provide the greatest host benefits. However, utilizing this knowledge is much more difficult than obtaining it.

Influencing the AMF community is a difficult challenge. Utilizing feedbacks to control feedbacks requires significantly more knowledge about plant-AMF interactions than is currently available. Because host plants usually support an entire community of AMF simultaneously, it remains unknown if favoring one AMF species over another can shift competitive interactions to an extent that a native plant is facilitated and an invader

is restricted. It is further unknown how such a feat might be accomplished at a specific invaded location. Another obstacle is the fate of the number of native species that likely share preference for and dependence on AMF species that facilitate invaders. Given the extraordinary diversity of plant-AMF interactions and the extremely limited knowledge on specific plant-AMF responses to these interactions, plant-AMF associations most likely exist that favor native plants over invaders. If we can identify and exploit these relationships then we might enhance restoration success.

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Chapter 2. Seasonal variation in arbuscular mycorrhizal fungi root colonization of cheatgrass (*Bromus tectorum* L.), an invasive winter annual

Introduction

Cheatgrass (*Bromus tectorum* L.) is a highly invasive introduced winter annual grass in western North America (Knapp 1996). Cheatgrass is very successful at colonizing new sites due to its ability to germinate in autumn when most native vegetation is senescing, remain active throughout the winter when conditions allow, utilize soil moisture before most native vegetation resumes growth in the spring, and reproduce and die when soil moisture is exhausted (Stewart and Hull 1949; Klemmedson and Smith 1964). Cheatgrass is described as a facultative mycotroph (Goodwin 1992), and has been associated with reduced AMF density in invaded soils (Al-Qarawi 2002). Native grasses exhibited lower diversity of AMF species colonizing their roots when grown with cheatgrass than without (Hawkes et al. 2006). Mycorrhizal colonization has reduced cheatgrass biomass (Rowe et al. 2007) or had no effect (Allen and Allen 1982).

Mycorrhizal colonization of roots is highly seasonal, with colonization lowest at cool temperatures, and highest at warm temperatures (Rabatin 1979; Bentivenga and Hetrick 1992; Sanders and Fitter 1992; Lutgen et al. 2003). Generally, this seasonality coincides with host activity, as most plant species are senescent or dormant at cool

temperatures, and activity is highest during periods of warm temperatures. This correlation with host activity was further shown in colonization differences between perennial cool- and warm-season grasses at the same site, where cool-season grass colonization peaked in late spring and early autumn and warm-season grass colonization peaked in mid-summer (Bentivenga and Hetrick 1992).

The only winter annual grasses investigated for AMF colonization during the winter are the winter cereal crops wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and rye (*Secale cereale* L.) (Jakobsen and Nielsen 1983). Several studies indicate that winter wheat is not colonized until spring (Hetrick et al. 1984; Mohammad et al. 1998), but others have shown winter colonization (Buwalda et al. 1985; Dodd and Jeffries 1986). Hetrick and Bloom (1984) found almost no colonization of winter wheat at 10° C, less than 1% colonization at 15° C, with peak colonization occurring between 20° and 25° C.

The goal of this study was to determine how AMF colonization changes over time in cheatgrass roots. This study tested the hypothesis that cheatgrass roots are colonized by AMF throughout its lifespan in invaded soils. Because cheatgrass is a winter annual and uses this strategy to gain an advantage over native vegetation, it is important to know how AMF are involved with cheatgrass throughout its life.

Materials and Methods

Patches containing dense cheatgrass populations the prior year were identified (through standing dead shoots) during the summer of 2007 in an invaded shortgrass steppe plant community at the Pine Ridge Natural Area (Fort Collins, CO, USA). Two random patches were selected approximately 150 m apart and monitored until cheatgrass

seedlings emerged in autumn. The north site (40.5489°, -105.1435°) is located on an east-facing slope, on a soil classified as a hilly Haplustoll (USDA 1980). The south site (40.5473°, -105.1428°) is located on a rocky, southeast-facing hillside, on a Satanta loam (Fine-loamy, mixed, superactive, mesic Aridic Argiustolls) (USDA 1980). Six weeks after germination was estimated to have occurred, five cheatgrass individuals from each patch were excavated and their roots were washed, harvested, and stored in 70% ethanol. Approximately every 3 weeks after this initial harvest, an additional five cheatgrass individuals were collected in the same manner from each patch. Collection was repeated until cheatgrass individuals began to senesce in the spring. Soil temperature data was obtained from the Colorado State University Fort Collins Weather Station (approximately 5.5 km from study sites) at their website:
http://climate.colostate.edu/~autowx/fclwx_about.php (Accessed 7-11-2008).

Root samples were washed, cleared in 2.5% KOH for 30 min. at 90 °C, rinsed, acidified in 1% HCl for 4 h., stained in acid glycerol containing 0.05% trypan blue for 30 minutes at 90 °C and destained in acid glycerol for 30 minutes at 90 °C (Koske and Gemma 1989). Root samples were divided into three subsamples each. Each subsample was observed under 400 X magnification and the presence of hyphae, vesicles, and arbuscules were determined using 100 root intersections per subsample with a crosshair reticle (McGonigle et al. 1990).

Colonization count data were arcsine square root transformed, and normality was confirmed through residuals plots, box plots and Wilks-Shapiro normality tests. Colonization data were analyzed using the proc mixed random effects model in SAS version 9.1 (The SAS Institute, Cary, NC, USA), with site, date, their interaction,

subsamples nested within samples, and samples nested within sites as effects. Nesting of samples and subsamples was incorporated to minimize pseudoreplication, with treatments applied at the site level and samples (individual plants) treated as independent replicates within each site.

Results

A total of 11 sampling dates occurred between October 2007 and June 2008, giving a total of 110 individual samples and 330 subsamples. Cheatgrass roots were colonized during every sampling date (Figure 1). Subsample vesicle colonization ranged from 0% to 7%, arbuscule colonization ranged from 0% to 10%, hyphal colonization ranged from 0% to 33%, and total colonization ranged from 0% to 34%. Means (and standard errors) for all samples and subsamples across all sampling dates and both sites were: mean vesicle % colonization: 0.93 (0.08), mean arbuscular % colonization: 1.89 (0.12), mean hyphal % colonization: 8.04 (0.34), and total % colonization: 8.35 (0.35). Vesicle, arbuscule, and hyphal percent colonization do not sum to the total percent colonization because one colonized intersection was often colonized by multiple structures, but the intersection only contributed 1% to the total percent colonization. Colonization decreased significantly during the month of January, and did not begin to increase until soil temperature began to warm in March (Figure 1).

Peak colonization occurred during the May 17 sampling interval, when florets were produced, and colonization decreased in the subsequent June 8 sampling interval, when senescence of cheatgrass was occurring (Figure 1). However, vesicle colonization was still increasing during this final sampling interval. Population variance was not

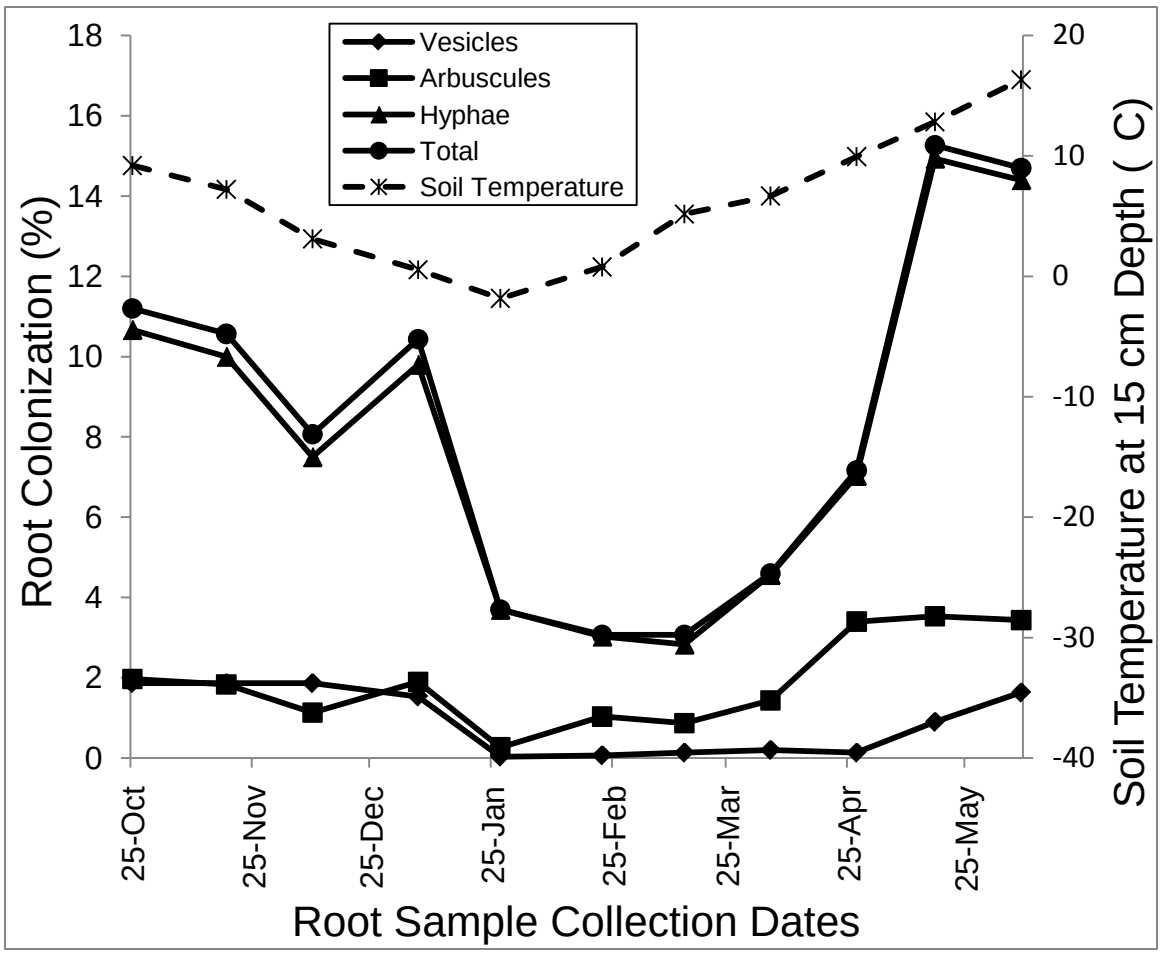

Figure 1. Percent colonization of cheatgrass roots by AMF structures across the lifespan of cheatgrass collected from two patches in an invaded shortgrass prairie near Fort Collins, CO, USA. Left y-axis gives percent colonization for vesicles, arbuscules, hyphae, and total AMF colonization. Vesicle, arbuscule, and hyphal percent colonization do not sum to the total percent colonization because one colonized intersection was often colonized by multiple structures, but the intersection only contributed 1% to the total percent colonization. Right y-axis gives soil temperature at a depth of 15 cm, measured at the Colorado State University Fort Collins Weather Station, approximately 5.5 km from the study sites.

affected by variance of samples within patches or subsamples within samples (Table 1). Population variance in AMF colonization was due entirely to site and date effects, and their interaction (Table 1). Sampling date was significant for all colonization structures, but site was only significant for arbuscule colonization (Table 1). South site mean

arbuscule colonization (and standard error) was 1.37% (0.14), while the north site mean arbuscule colonization (and standard error) was 2.41% (0.18). The interaction between site and date had a significant effect on population variance for all colonization structures (Table 1).

Table 1. Analysis of variance for effects of date, site, sample, subsample, and date x site interaction on percent root colonization of cheatgrass by AMF structures.

	Colonization Structure							
	Vesicles				Arbuscules			
Effect	Variance Component	Error df	F	p	Variance Component	Error df	F	p
Date	0.002	10	6.88	**0.003**	0.002	10	2.98	**0.050**
Site	0.000	9	0.96	0.354	0.001	10	7.99	**0.019**
Sample(Site)	< 0.001	290	1.98	0.097	< 0.001	290	1.32	0.263
Subsample(Sample)	0.000	290	0.94	0.501	0.000	290	0.50	0.889
Date x Site	< 0.001	290	3.08	**0.001**	0.001	290	4.86	< **0.001**
Residual	0.003				0.005			
	Hyphae				Total			
Effect	Variance Component	Error df	F	p	Variance Component	Error df	F	p
Date	0.006	10	4.88	**0.010**	0.006	10	4.99	**0.009**
Site	< 0.001	10	2.83	0.122	< 0.001	11	2.62	0.134
Sample(Site)	< 0.001	290	1.54	0.191	< 0.001	290	1.99	0.096
Subsample(Sample)	0.000	290	0.62	0.797	0.000	290	0.46	0.916
Date x Site	0.003	290	7.48	< **0.001**	0.003	290	7.11	< **0.001**
Residual	0.006				0.006			

Discussion

While site was significant for arbuscule colonization, the ecological significance of a 1% difference in arbuscule colonization is probably small. The significant site x date interaction was most likely due to the difference between sites at the end of the sampling effort. The north site population senesced earlier than the south site population, and during the final sampling the south site individuals were much greener than the north site

individuals, as cheatgrass turns purple following seed set. This resulted in a much greater reduction in arbuscular, hyphal and total colonization during the final sampling interval at the north site, and much greater vesicle colonization at the north site, relative to the south site.

As vesicles are C storage structures for the AMF, an increase in vesicles during host senescence indicates the AMF were storing increasing levels of C as the C supplied by the host plant began to diminish, most likely to increase spore production once the C source began to diminish. Bentivenga and Hetrick (1992) observed high colonization of perennial grass roots long after senescence of the host plant, and speculated that at the later stages of host growth, AMF may turn parasitic in order to maximize C availability for sporulation.

AMF colonization of host roots has been previously observed to decrease significantly at 15° C, and stop almost completely at 10 °C (Liu et al. 2004). We observed a similar trend for colonization of cheatgrass roots, and increases in colonization occurred only after the soil temperature rose above 10 °C at the 15 cm depth in spring (Figure 1). The reduction in colonization at low temperatures is thought to result from reduced C supplied by the plant host (Gavito et al. 2005). Their study found that C supply was reduced at low temperatures while P transfer from AMF to the plant host remained constant at temperatures between 10 and 25 °C. However, other studies have shown that AMF colonization reduces plant growth at low temperatures (Liu et al. 2004) due to the AMF consuming host C at low temperatures but not providing P to the host (Hetrick and Bloom 1984). Because of the variation in interactions between plant

species and different AMF hosts (Gustafson and Casper 2006), it is possible that certain AMF species are more parasitic than others on cheatgrass at low temperatures.

Cheatgrass invasion often completely overwhelms native vegetation, creating virtual monocultures that exclude other plant species (Knapp 1996). Cheatgrass-invaded soils have been observed to contain lower AMF density than soils dominated by native vegetation (Al-Qarawi 2002). The native vegetation that is replaced by cheatgrass includes many perennial species that are active throughout the summer and dormant through the winter, including shrubs, forbs and C_4 grasses. Large differences in AMF species sporulation and abundance have been observed in single host plant species during the growing season, and between C_3 and C_4 grasses both within and between sampling dates (Bentivenga and Hetrick 1992). Many of the AMF species adapted to growth during warm soil temperatures with hosts active during this period would not have access to a suitable host in soils where cheatgrass is the only host, as cheatgrass would be senescing while they are becoming active. Thus, the loss of seasonal host activity due to cheatgrass dominance most likely favors AMF species adapted to early season growth. However, because cheatgrass does not appear to ever attain high colonization by AMF, it does not appear to be a superior host for these species either. These factors most likely contribute to loss of AMF density, and probably a loss of AMF species diversity, which could hinder establishment of native plant species, many of which are more dependent on AMF.

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Chapter 3. Arbuscular mycorrhizal fungal community differs between a coexisting native shrub and introduced annual grass

Introduction

Feedbacks between non-native plants and the soil microbial community have been implicated in successful plant invasions (Klironomos 2002; Callaway et al. 2004; Jordan et al. 2008). These feedbacks, particularly between plants and arbuscular mycorrhizal fungi (AMF), have been shown to occur either through increased competitive abilities of non-native invaders provided by AMF (Marler et al. 1998; Zabinski et al. 2002; Bray et al. 2003; Stampe and Daehler 2003; Harner et al. 2010), or through alterations to the AMF community that favor the invader (Greipsson and DiTommaso 2006; Hawkes et al. 2006; Stinson et al. 2006; Callaway et al. 2008; Vogelsang and Bever 2009). Vogelsang and Bever (2009) concluded that these alterations may increase invader persistence. However, due to the variation in interactions between AMF and plant species (Sanders and Fitter 1992; Johnson et al. 1992; Wilson and Hartnett 1998; Vandenkoornhuyse et al. 2003; Gustafson and Casper 2006), it is unknown if AMF community changes due to plant invasion differ significantly from native vegetation, or if they simply reflect variation among plant hosts. Because of the influence of extrinsic factors, such as environment (Allen et al. 1995; Opik et al. 2006; Ji et al. 2010), land use intensity (Oehl

et al. 2003; Rosendahl and Matzen 2008), grazing (Eom et al. 2001; Murray et al. 2010), fire (Wicklow-Howard 1989; O'Dea 2007), neighbor identity (Hausmann and Hawkes 2009), etc. on AMF communities, comparing changes to AMF communities due to invaders with AMF communities associated with natives becomes difficult. To determine ecological differences between introduced and native species, native species need to be carefully selected to elucidate invader differences that are ecologically relevant (van Kleunen et al. 2010).

Invasive grasses are particularly destructive, as they are globally invasive and alter ecosystem processes (D'Antonio and Vitousek 1992). Cheatgrass (*Bromus tectorum* L.) invasion in the semi-arid sagebrush (*Artemisia* spp.) rangelands of western North America has caused a catastrophic shift from shrub-dominated plant communities to those dominated by an introduced annual grass. Sagebrush historically occurred on over 60 million hectares in western North America, and big sagebrush (*Artemisia tridentata*) was dominant or co-dominant with perennial grasses over much of this area (West 1983). Big sagebrush is considered a foundation species across much of the intermountain west (Prevey et al. 2010). Historically, sagebrush steppe vegetation comprised greater than 400,000 km^2 in the intermountain west, including parts of 11 U.S. states and 1 Canadian province (Mack 1981). Cheatgrass has invaded 200,000 km^2 in the intermountain west (Mack 1989), and maintains its dominance through its alteration of the fire regime (Klemmedson and Smith 1964). Cheatgrass recovers rapidly following fire, but native species such as big sagebrush are not adapted to the reduced fire return intervals associated with cheatgrass invasion (Young and Evans 1978). Historic fire return interval estimates range from 60 to 110 years across the sagebrush steppe (Whisenant

1990), to as high as 100 to 240 years for stands of Wyoming big sagebrush (Baker 2006). Cheatgrass invasion has reduced these intervals to 3 to 5 years (D'Antonio and Vitousek 1992). Cheatgrass often attains nearly 100% coverage in invaded areas, and approximately 20% of the historic sagebrush steppe is dominated by cheatgrass, which restricts establishment of native vegetation (Knapp 1996).

The impacts of cheatgrass invasion on the AMF community are not well known. Cheatgrass is a facultative associate of AMF (Allen 1984). Cheatgrass has been shown to reduce the AMF diversity of neighboring vegetation (Hawkes et al. 2006), and soils from cheatgrass dominated areas have lower mycorrhizal inoculum than uninvaded soils (Al-Qawari 2002). However, the identities of AMF species associating with cheatgrass, as well as the diversity of cheatgrass-AMF associations, are largely unknown. On the other hand, big sagebrush has been shown to have high diversity of associated AMF (Allen et al. 1995). An important question in understanding replacement of big sagebrush dominance by cheatgrass is how this shift alters the diversity of AMF in invaded sagebrush steppe habitat, thereby impacting future recovery and restoration of invaded communities. The goal of this research was to determine how coexisting cheatgrass and big sagebrush differ in their associations with AMF, and identify AMF species that may be important for restoration of big sagebrush in cheatgrass-invaded soils. The hypothesis tested was that alpha diversity of AMF associated with cheatgrass is lower than alpha diversity of AMF associated with big sagebrush. AMF species were identified from three locations in Colorado, Utah and Wyoming USA (hereafter the CO, UT and WY sites, respectively), using trap cultures with field-collected soil and root material grown for 1 year in a greenhouse.

Materials and Methods

Study Sites

Three sites were selected that contain coexisting cheatgrass and big sagebrush (Table 1). By selecting study sites where the target host plant species are interspersed, site effects and previous disturbances that alter the AMF community were minimized.

Table 1. Characteristics of sites where soil samples were obtained for culturing.

Site	Location	Elevation	Soil Texture[1]	Soil Classification[1]	Landscape Position	Community Age
CO	39.9063°, -108.3970°	1990 m	loam	Fine-loamy, mixed Borollic Camborthids	basin floor	Mature sagebrush steppe
UT	40.4558°, -112.0484°	1950 m	cobbly sandy clay loam	Clayey-skeletal, smectitic, frigid Lithic Argixerolls	rocky, upland slope	Intermediate sagebrush steppe
WY	42.2557°, -104.7671°	1340 m	sandy loam	Coarse-loamy, mixed, superactive, calcareous, mesic Ustic Torrifluvents	bottom-land floodplain	Immature sagebrush steppe

[1]Soil series data were obtained using NRCS Web Soil Survey http://websoilsurvey.nrcs.usda.gov/app/HomePage.htm

These sites are geographically separated and represent three distinct levels of plant community age (Table 1). The WY site is near the eastern edge of big sagebrush's present range. Five years before sampling, a 10 ha grazing exclosure was constructed, allowing big sagebrush and other native vegetation to re-establish. Dominant vegetation included cheatgrass, needle and thread grass (*Hesperostipa comata* (Trin. & Rupr.) Barkworth), deathcamas (*Zigadenus venenosus* S. Watson), silver sagebrush (*Artemisia cana* Pursh), and Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis* Beetle & Young) based on visual cover estimates at the time of sampling (data not

shown). The UT site contained a diverse flora dominated by native vegetation that is managed using controlled burns to reduce the dominance of woody vegetation. Dominant plant species included Gambel oak (*Quercus gambelii* Nutt.), bulbous bluegrass (*Poa bulbosa* L.), arrowleaf balsamroot (*Balsamorhiza sagittata* (Pursh) Nutt.), Wyoming big sagebrush, cheatgrass, and antelope bitterbrush (*Purshia tridentata* (Pursh) DC). The CO site is located in the Piceance Basin. Dominant vegetation included Wyoming big sagebrush, western wheatgrass (*Pascopyrum smithii* (Rydb.) A. Löve), muttongrass (*Poa fendleriana* (Steud.) Vasey), cheatgrass, and *Alyssum* spp.

Sample Collection

To compare AMF associations of cheatgrass and big sagebrush, 16 individuals of each host at each site were selected and excavated. Peak colonization of big sagebrush roots was previously identified as occurring in late spring (Trent et al. 1994). The timing of peak AMF colonization of cheatgrass was assumed to be similar to other C_3 grasses and occur just before flowering in late spring (Bentivenga and Hetrick 1992). Sampling occurred from May 19-22, 2008, targeting the estimated peak colonization interval for both hosts. Because these plant species differ drastically in their growth strategies, juveniles of big sagebrush less than 15 cm in height were selected to more appropriately compare a long-lived shrub to a winter annual grass, as juveniles would be much closer in age, mass, height, root volume and C fixation capacity to cheatgrass. To minimize the effects of one host on the other, individuals to be sampled were selected that were at least 50 cm from individuals of the other host. Sixteen interspersed individuals of each host plant species fitting the above criteria across each selected study site were identified. Soil and roots were excavated and removed from a depth and diameter of 15 cm

immediately around each of the individuals. Soils were placed in sterile bags and kept cool for transport to the greenhouse.

Culturing of AMF

Soils were used to establish trap cultures for propagation of AMF following Stutz and Morton (1996). Each of the 96 soil samples was mixed 1:1 (v:v) with sterile sand, placed in 2.8 L pots, seeded with 80 to 100 surface-sterilized seeds (5 min. in 5% sodium hypochlorite solution) of Sudangrass (*Sorghum bicolor* (L.) Moench ssp. *drummondii* (Nees ex Steud.) de Wet & Harlan) and placed in a climate-controlled glasshouse at Colorado State University, Fort Collins. The glasshouse was maintained at 20º C nighttime and 24º C daytime temperatures and supplemented with sodium vapor lights to maintain 16 hours of daylight. Cultures were watered daily and re-randomized on the bench every 2 weeks to minimize microclimate effects. Cultures were grown for 120 days and watering was then stopped to allow soils to dry slowly for induction of sporulation. After 14 days of drying in low-light conditions, Sudangrass shoots were removed and a 250 ml subsample of soil was collected. Sterile sand was used to fill the voids left by sample collection. The cultures were then reseeded with 80 to 100 surface-sterilized Sudangrass seeds, and culturing was repeated. This process was repeated for three rounds of culturing to maximize sporulation of AMF species present (Stutz and Morton 1996).

Identification of AMF

AMF spores were isolated from subsamples of each culture for each culturing interval using sucrose density gradient centrifugation (Daniels and Skipper 1982). A 50-cm3 subsample of soil removed at each sampling interval was passed through 500 µm

and 38 µm sieves using a water drench. Material retained by the 38-µm sieve was transferred to a 50 ml centrifuge tube containing a 20/60% sucrose gradient and centrifuged at 1000 x g for 1 minute. The supernatant was poured into a 38-µm sieve and thoroughly rinsed. The rinsed material retained on the 38-µm sieve was transferred to a glass Petri dish, and spores were isolated and removed under a dissecting microscope using manually extended glass pipette tips. Isolated spores were mounted in polyvinyl lacto-glycerol and Meltzer's reagent (Koske and Tessier 1983; Stutz and Morton 1996) and species were identified using spore wall characteristics (Morton 1988). Mounted spores were compared to voucher specimens at the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM, West Virginia University, Morgantown) and to published descriptions of AMF species.

Analyses of Data

AMF species occurring in each culture were analyzed by first comparing AMF alpha diversity between hosts at each study site and between hosts across study sites using t-tests on square-root transformed richness data. Mean richness per culture was also used to calculate alpha diversity for each site across hosts and total diversity for the study. Total richness across samples was then used to calculate gamma diversity for each site-host combination, each host across sites, each site across hosts, and total diversity for the study. The alpha- and gamma-diversity calculations were then used to calculate respective beta diversities using the equation $\beta = (\gamma/\alpha)-1$ (Whittaker 1972). Site and host effects and their interaction on AMF composition were analyzed using multivariate analyses of variance (MANOVA) with SAS version 9.1 (The SAS Institute, Cary, NC, USA). Community composition was compared between hosts at each site and across

sites using multiple response permutation procedure (MRPP) with 4,999 permutations in PC-ORD version 5.31 (MjM Software, Gleneden Beach, OR, USA). To identify potential AMF species associations with a particular host within and across sites, indicator species analysis was conducted using PC-ORD with 4,999 permutations.

Results

All 96 cultures contained AMF species, with a range from 1 to 12 species. A total of 32 AMF species were isolated from the 96 trap cultures, including 7 unidentified species that were isolated across all 3 sites (Figure 1). *Glomus mosseae* was the most common species observed in the study, occurring in 83 of the 96 cultures across all sites (Figure 1). Other common species associating with both hosts and occurring at all three sites were *Glomus aggregatum*, *Glomus claroideum*, *Glomus eberneum*, *Glomus intraradices*, *Glomus versiforme*, *Glomus viscosum*, *Diversispora spurca*, *Paraglomus occultum* and *Entrophospora infrequens* (Figure 1). Two species were common in only 2 of the 3 sites: *Archaeaospora trappei* and *Glomus constrictum* (Figure 1). *Acaulospora delicata* (WY site) and *Pacispora scintillans* (UT site) were moderately common at only 1 site and not isolated elsewhere. Many isolated AMF species (16 of the 32 total species isolated) occurred in less than 5% of the cultures.

Certain AMF species appeared to preferentially associate with one host over another (Figure 1). Some of these associations were consistent across sites where they occurred, while others did not produce a consistent trend across sites. For instance, *Diversispora spurca* occurred much more frequently in cheatgrass cultures (81%) compared to big sagebrush (44%) at the WY site, was much more prevalent in big sagebrush cultures

Figure 1a.

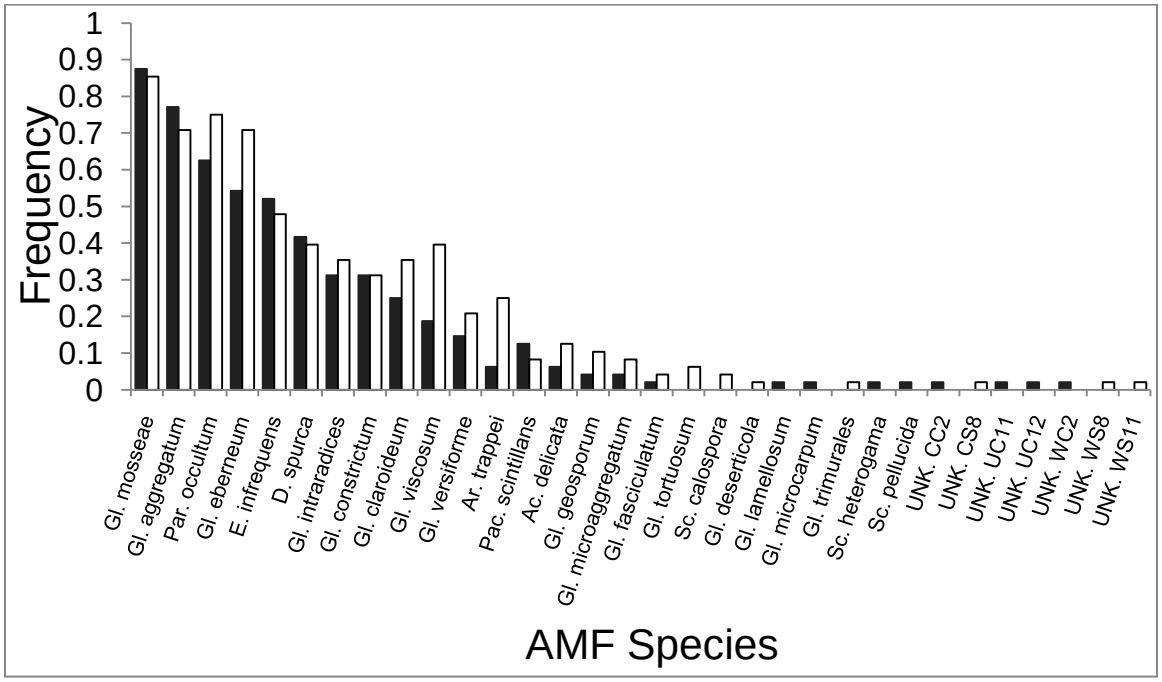

Figure 1b.

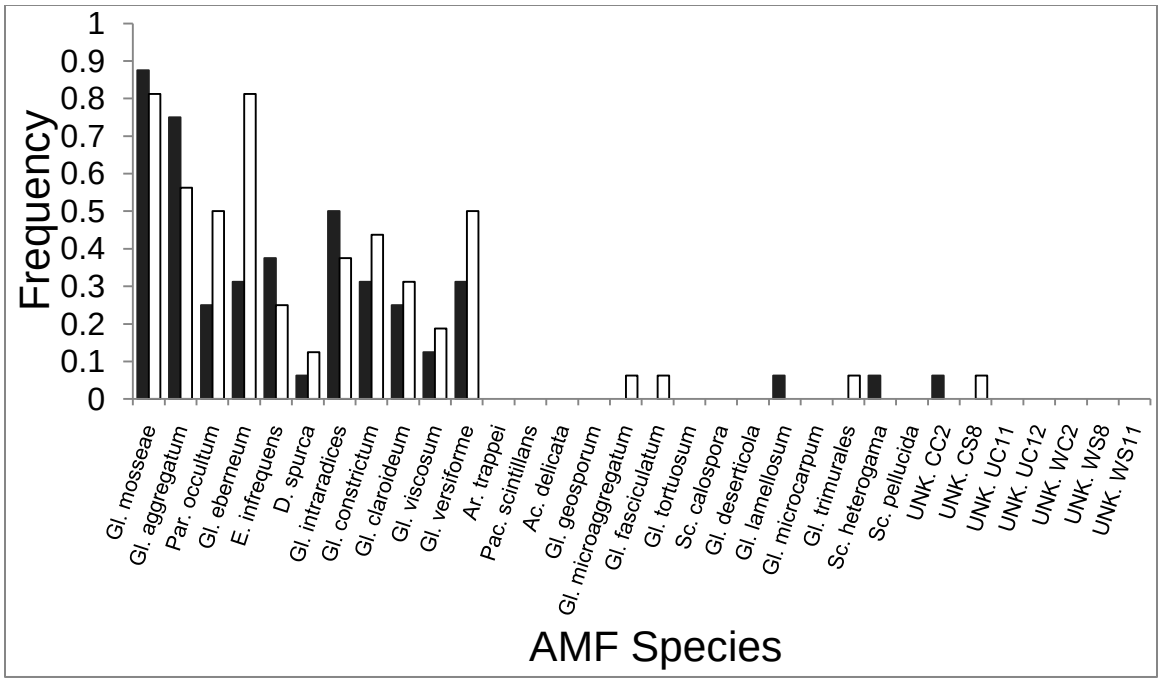

Figure 1c.

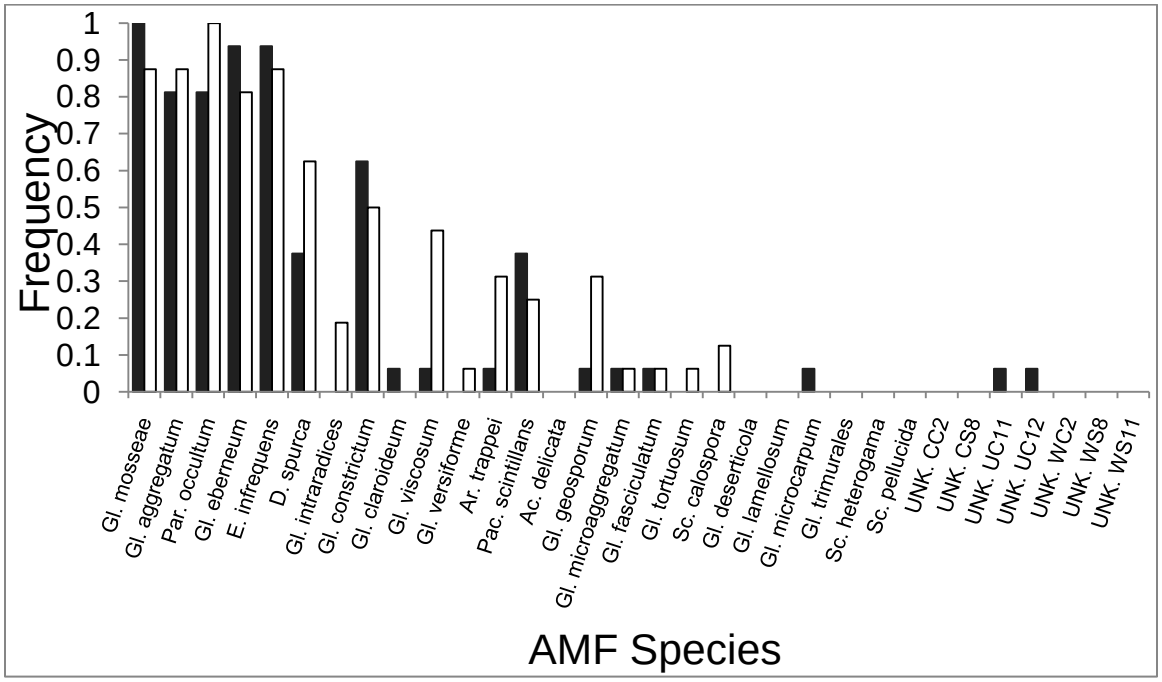

Figure 1d.

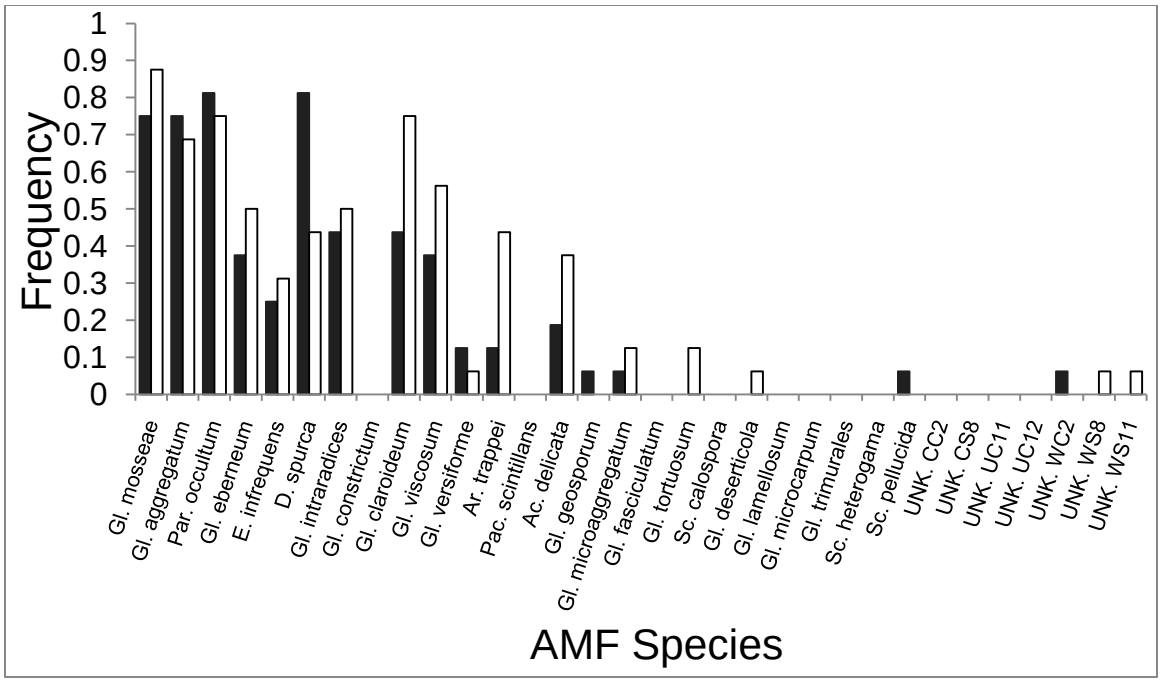

Figure 1. Frequency of AMF species occurring in trap cultures associated with each host plant at each site. Ac. = Acaulospora, Ar. = Archaeospora, D. = Diversispora, E. = Entrophospora, Gl. = Glomus, Pac. = Pacispora, Par. = Paraglomus, Sc. = Scutellospora.

a. Total frequency of AMF species across all three sites, based on identification of spores produced in 48 trap cultures using soil samples for each host plant species. Black bars indicate cheatgrass associations; white bars indicate big sagebrush associations.

b. Total frequency of AMF species at the Colorado site, based on identification of spores produced in 16 trap cultures for each host plant species. Black bars indicate cheatgrass associations; white bars indicate big sagebrush associations.

c. Total frequency of AMF species at the Utah site, based on identification of spores produced in 16 trap cultures for each host plant species. Black bars indicate cheatgrass associations; white bars indicate big sagebrush associations.

d. Total frequency of AMF species at the Wyoming site, based on identification of spores produced in 16 trap cultures for each host plant species. Black bars indicate cheatgrass associations; white bars indicate big sagebrush associations.

(63%) compared to cheatgrass (38%) at the UT site, and was only slightly more frequent in big sagebrush cultures (13%) than cheatgrass (6%) at the CO site.

AMF diversity measures are presented in Table 2. Diversity measures are as follows: α diversity is the species richness per sample, presented as mean richness in replicate culture samples (host and site as groups); γ diversity is the total richness across sample units, presented as total species richness across replicate culture samples (host and site as groups); and β diversity is the rate of change between replicate culture samples (i.e. number of unique communities) in a group of replicate samples (host and site as groups), calculated here as $\beta = (\gamma/\alpha)-1$ (Whittaker 1972). Alpha diversity of AMF species was higher with big sagebrush as host compared to cheatgrass at all sites (Table 2). However, only the statistical comparison across all sites indicated a significant difference in α diversity (Table 3). Gamma diversity was almost identical between host plant species within and across sites, although the CO site had the lowest gamma

Table 2. Diversity of arbuscular mycorrhizal fungi within and across sites and plant hosts.

	Plant Host											
	Cheatgrass				Big Sagebrush				Total			
		Diversity				Diversity				Diversity		
Site	N	α[1]	β[2]	γ[3]	N	α	β	γ	N	α	β	γ
CO[4]	16	4.3	2.3	14	16	5.1	1.9	15	32	4.7	2.8	18
UT	16	6.4	1.7	17	16	7.4	1.3	17	32	6.9	2.0	21
WY	16	5.7	1.8	16	16	6.7	1.5	17	32	6.2	2.2	20
All Sites	48	5.5	3.5	25	48	6.4	2.8	24	96	6.0	4.3	32

[1]Alpha diversity, calculated as mean AMF species richness per trap culture.
[2]Beta diversity, calculated as $\beta = (\gamma/\alpha)-1$ *(Whittaker 1972).*
[3]Gamma diversity, calculated as total AMF species richness across samples.
[4]CO, UT and WY are the Colorado, Utah and Wyoming, USA, sites, respectively.

Table 3. Results of t-tests for α diversity of arbuscular mycorrhizal fungi isolated from trap cultures associated with cheatgrass or big sagebrush within and across study sites (α=0.05). Alpha diversity was calculated as mean AMF species richness per trap culture.

Site	df	t Value	Pr > |t|
CO[1]	30	1.65	0.109
UT	30	1.66	0.107
WY	30	1.53	0.135
All Sites	94	2.46	0.016

[1]CO, UT and WY are the Colorado, Utah and Wyoming, USA, sites, respectively.

diversity (Table 2). Beta diversity was higher with cheatgrass as host compared to big sagebrush within and across all sites (Table 2).

Site had a significant effect on AMF community composition ($p < 0.0001$), while host did not ($p = 0.0614$), and the interaction between site and host had a significant effect on AMF community composition ($p = 0.0268$) (Table 4). Because of the significance of the interaction effect on AMF community composition and low p-value of the non-significant host effect, the effect of host at each site on AMF community composition was analyzed using MRPP (Table 5). Only the UT site ($p = 0.013$) indicated

Table 4. Two-way multivariate analysis of variance testing significance of site, host and site x host interaction effects on arbuscular mycorrhizal fungi communities isolated from trap cultures.

Effect	Num df	Den df	Wilks' Lambda[1]	F-Value	Pr > F
Site	62	120	0.0604	5.94	< 0.0001
Host	31	60	0.5486	1.59	0.0614
Site x Host	62	120	0.3147	1.51	0.0268

[1]A Wilks' Lambda close to zero indicates a strong relationship, close to 1 indicates a weak relationship.

Table 5. Multiple response permutation procedure with 4,999 permutations for the effects of host plant species on AMF communities isolated from trap cultures from three sites.

Site	T[1]	A[2]	P
CO[3]	-1.688	0.022	0.063
UT	-2.806	0.033	0.013
WY	-1.142	0.014	0.128
Total	-1.142	0.004	0.128

[1]Test Statistic. T describes degree of group separation. A negative T close to zero indicates little separation. A negative T much lower than zero indicates strong separation.
[2]Agreement Statistic. A describes within-group separation. When A = 1, all observations are identical. When A = 0, within-group heterogeneity equals what is expected due to chance.
[3]CO, UT and WY are the Colorado, Utah and Wyoming, USA, sites, respectively.

an effect of host on AMF communities (Table 5), which explained the significant interaction effect found in the MANOVA. Indicator species analysis was used to determine if any of the AMF species were more likely to associate with one host over another. No AMF species were found to have a higher frequency with cheatgrass, but two AMF species (*Archaeospora trappei* and *Glomus viscosum*) occurred more frequently with big sagebrush compared to cheatgrass (Table 6).

Table 6. Indicator species[1] analysis of arbuscular mycorrhizal fungi species isolated from cheatgrass and big sagebrush trap cultures with 4,999 permutations.

AMF Species	Maximum Indicator Group[2]	Indicator Value[3]	p value
Archaeospora trappei (R.N. Ames & Linderman) J.B. Morton & D. Redecker	Artemisia	20.0	**0.0238**
Glomus viscosum T.H. Nicolson	Artemisia	26.9	**0.0444**
Glomus eberneum L.J. Kenn., J.C. Stutz & J.B. Morton	Artemisia	40.1	0.1390
Glomus tortuosum N.C. Schenck & G.S. Sm.	Artemisia	6.2	0.2350
Paraglomus occultum (C. Walker) J.B. Morton & D. Redecker	Artemisia	40.9	0.2721
Glomus claroideum N. C. Schenck & G. S. Sm.	Artemisia	20.8	0.3673
Glomus geosporum C. Walker	Artemisia	7.4	0.4367
Acaulospora delicata C. Walker, C.M. Pfeiff. & Bloss	Artemisia	8.3	0.4833
Scutellospora calospora (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders	Artemisia	4.2	0.5021
Glomus versiforme (P. Karst.) S.M. Berch	Artemisia	12.3	0.5941
Glomus aggregatum N.C. Schenck & G.S. Sm.	Bromus	40.2	0.6437
Glomus microaggregatum Koske, Gemma & P.D. Olexia	Artemisia	5.6	0.6829
Pacispora scintillans (S.L. Rose & Trappe) C. Walker, Vestberg & Schuessler	Bromus	7.5	0.7443
Glomus intraradices N.C. Schenck & G.S. Sm.	Artemisia	18.8	0.8262
Entrophospora infrequens (I.R. Hall) R.N. Ames & R.W. Schneid.	Bromus	27.1	0.8458
Diversispora spurca (C.M. Pfeiff., C. Walker & Bloss) C. Walker & A. Schuessler	Bromus	21.4	1.0000
Glomus constrictum Trappe (as *constrictus*)	Bromus	15.6	1.0000
Glomus deserticola Trappe, Bloss & J.A. Menge	Artemisia	2.1	1.0000
Glomus fasciculatum Gerd. & Trappe (as *fasciculatus*)	Artemisia	2.8	1.0000
Glomus lamellosum Dalpé, Koske & Tews	Bromus	2.1	1.0000
Glomus microcarpum Tul. & C. Tul. (as *microcarpus*)	Bromus	2.1	1.0000
Glomus mosseae Gerd. & Trappe	Bromus	44.3	1.0000
Glomus trimurales Koske & Halvorson	Artemisia	2.1	1.0000
Scutellospora heterogama (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders	Bromus	2.1	1.0000
Scutellospora pellucida (T.H. Nicolson & N. C. Schenck) C. Walker & F.E. Sanders	Bromus	2.1	1.0000
UNK CC2	Bromus	2.1	1.0000
UNK CS8	Artemisia	2.1	1.0000
UNK UC11	Bromus	2.1	1.0000
UNK UC12	Bromus	2.1	1.0000
UNK WC2	Bromus	2.1	1.0000

Table 6 (continued).

AMF Species	Maximum Indicator Group[2]	Indicator Value[3]	p value
UNK WS8	Artemisia	2.1	1.0000
UNK WS11	Artemisia	2.1	1.0000

[1] An indicator species is ideal if it occurs in all samples of one group, and none of another group. Indicator species are calculated by multiplying the proportional abundance of a species in a group relative to its abundance in the other group by the proportional frequency of the species in each group.
[2]Maximum Indicator Group indicates the group that each species is most abundant in.
[3]An Indicator Value of 100 equals perfect indication of maximum indicator group.

Discussion

This study compared AMF communities associated with coexisting big sagebrush and cheatgrass from three sites. We found that alpha diversity of AMF associated with cheatgrass is lower than alpha diversity of AMF associated with big sagebrush. Across study sites, alpha diversity of AMF species was significantly higher in big sagebrush cultures compared to cheatgrass. Although at the individual site level the alpha diversity comparisons were not different (p-values ranged from 0.107 to 0.135), all three sites showed the same trend. Mean AMF species richness per sample in big sagebrush cultures was close to one AMF species higher than in cheatgrass cultures. This represents about a 15% reduction in species richness associated with cheatgrass in all sites.

Two AMF species, *Archaeospora trappei* and *Glomus viscosum*, were more prevalent in cultures from big sagebrush compared to cheatgrass across multiple sites. These indicator species for big sagebrush may be important to big sagebrush juveniles, but the present work did not separate the effects of individual AMF species on host

growth and survival. Further, while *Glomus viscosum* occurred at all three sites, *Archaeospora trappei* was only found at two. So, site effects may also be an important consideration for which AMF species have a strong association with big sagebrush. A previous AMF diversity study that focused solely on big sagebrush across multiple sites found that environmental conditions were more important than host in shaping the big sagebrush AMF community (Allen et al. 1995). Thus, the differences across sites not due to host are most likely a reflection of the sites themselves. Because our study used soil from underneath host plants, it is possible that the AMF community in each sample was not only influenced by the target host, but by neighboring vegetation and the legacy effects of prior inhabitants of that microsite (Hausmann and Hawkes 2009; Hausmann and Hawkes 2010). Future work should determine what the observed AMF community changes mean to big sagebrush in terms of AMF species loss, as AMF diversity and identity has a strong influence on plant community dynamics (van der Heijden et al. 1998; van der Heijden 2004). Furthermore, diversity measures of AMF in trap cultures are only presence/absence data, as sporulation rates associated with a trap culture may not be reflective of relative densities of AMF associating with the initial plant host in the field. A measure of relative densities between AMF species associating with specific hosts would be useful to compare individuals, populations and communities of plants.

Age of the plant community may also be important for AMF associations with big sagebrush. While our study lacks the necessary replication to determine the importance of community age, the mature sagebrush community exhibited the lowest alpha and gamma diversities and highest beta diversity for AMF. Due to the importance of plant diversity on AMF diversity (Eom et al. 2000; Hausmann and Hawkes 2010), the loss of

certain host plant species in mature sagebrush vegetation could lead to a loss of associated AMF species. Another consideration is that our big sagebrush samples only consisted of juveniles. Because conspecific woody vegetation associates with different AMF communities at different growth stages (Allen et al. 2003), the mature sagebrush community may have shifted to an AMF community better suited to the older individuals. If the difference in associations for different growth stages is considerable for big sagebrush, fewer available AMF species preferred by the juvenile sagebrush would then be available for the juveniles in this type of community, possibly affecting recruitment success and restoration of such sites. While densities of big sagebrush juveniles were not measured, locating an adequate number of juveniles for sampling in the mature sagebrush site was much more difficult than for the other sites (personal observation).

The calculated AMF beta diversities indicate a greater variability of AMF communities associated with cheatgrass than big sagebrush, while the gamma diversities of AMF are about equal between cheatgrass and big sagebrush. So, while both hosts associate with about as many AMF species at the individual site level and across sites, big sagebrush individuals can associate with more AMF species than cheatgrass individuals, and these sagebrush-associated AMF communities are more similar from one individual host to the next when compared to cheatgrass. Thus, AMF communities associated with big sagebrush at a given site are analogous diversity hotspots. The higher concentration of AMF species under big sagebrush is supported by the resource island effect of shrubs in arid and semi-arid ecosystems (Reynolds et al. 1999). These resource islands associated with shrubs support greater understory plant diversity in mature shrubs (Maestre and Cortina 2005), alter soil microbial diversity (Mummey and Stahl 2003;

Ewing et al. 2007), and harbor higher AMF propagules (Azcón-Aguilar et al. 2003; Camargo-Ricalde and Dhillion 2003) than shrub interspaces.

The study sites investigated here represent a best-case scenario with respect to cheatgrass invasion, where there still exists a major native plant component to the vegetation community. Differences observed under these circumstances will most likely become even more pronounced as the native vegetation is wholly replaced by dense stands of cheatgrass, and this shift persists over a long period of time. This shift has already occurred on over 20% of the historic sagebrush steppe plant community (Knapp 1996). However, interpreting differences and making meaningful inferences between severe cheatgrass infestations and uninvaded big sagebrush shrublands are difficult due to variance of sites and the importance of site history on both cheatgrass invasion and the AMF community. Overgrazing of native perennial grasses often allows cheatgrass to gain a foothold, and subsequent fires may remove remaining native vegetation (Klemmedson and Smith 1964; Young and Evans 1978; Knapp 1996). Both grazing (Eom et al. 2001; Murray et al. 2010) and fire (O'Dea 2007) alter the AMF community. Due to these confounding effects, this study investigated coexisting populations of cheatgrass and big sagebrush so that site differences between the populations could be minimized.

Invasion of shrublands by invasive grasses is a global ecological concern, causing significant alterations to native vegetation on multiple continents (D'Antonio and Vitousek 1992). One important consideration in the restoration of invasive grass-dominated shrublands is the length of time that remnant resource islands, including AMF diversity hotspots, remain intact when invasive grasses are the sole host. Knowledge of

this timeline is critical for successful restoration of native shrubland vegetation. Soil fertility differences associated with sagebrush resource islands have been shown to persist for 6 years (Bechtold and Inouye 2007), but differences in nutrient cycling were reduced within 14 years after shrub removal (Burke et al. 1987). The changes observed in our study where cheatgrass is only a minor component of the vegetation suggests that once cheatgrass becomes dominant and fires become prevalent, the loss of microbial hotspots could be rapid.

Due to the feedbacks occurring between different AMF species and plant hosts (Bever 2002), it is possible that certain AMF species critical to big sagebrush establishment could be less beneficial or even detrimental to cheatgrass. A reduction or loss of these AMF species due to cheatgrass invasion would further reduce big sagebrush recruitment. However, if these critical AMF species could be identified and promoted in invaded soils, successful restoration of big sagebrush could be more easily achieved. The differences in AMF species associations observed in this study may provide an initial assessment of the interactions needed to achieve this result.

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Chapter 4. Comparison of arbuscular mycorrhizal fungi associations between the invasive cheatgrass (*Bromus tectorum* L.) and native Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis* Beetle & Young)

Introduction

Arbuscular mycorrhizal fungi (AMF) have a strong influence on plant communities (Klironomos et al. 2000; van der Heijden et al. 1998a; van der Heijden et al. 1998b). Alternatively, plant communities can influence AMF communities (Eom et al., 2000; Hausmann and Hawkes, 2009). Within a site, diversity of AMF species can rival plant species diversity (Öpik et al. 2009), and co-existing plant species exhibit preferences for AMF (Vandenkoornhuyse et al. 2002; Vandenkoornhuyse et al. 2003). The diversity of interactions between plant hosts and fungal symbionts inspired the driver/passenger hypothesis, where certain AMF species are believed responsible for plant community changes, while other AMF species are the product of plant community changes (Hart et al. 2001). Numerous other factors also influence this relationship, including herbivory (Bethlenfalvay and Dekassian 1984; Eom et al. 2001; Klironomos et al. 2004; Bennett and Bever 2007; Murray et al. 2010), fire (Eom et al. 1999), soil disturbance (Moorman and Reeves 1979; Helgason et al. 1998; Oehl et al. 2003), nutrient deposition (Johnson 1993; Eom et al. 1999; Egerton-Warburton and Allen 2000; Egerton-

Warburton and Allen 2007), legacy effects of plant hosts (Hausmann and Hawkes 2010), and neighboring plants (Hausmann and Hawkes 2009). Because of the effects of this multitude of factors, AMF communities associated even with individual host plants vary widely among sites (Molina and Trappe 1978; Allen et al. 1995; Ji et al. 2010). Thus, attributing changes to AMF communities due to a single factor is difficult.

Interactions with the soil microbial community have been implicated in successful invasions by introduced plant species (Callaway et al. 2004; Wolfe and Klironomos 2005; Reinhart and Callaway 2006; van der Putten et al. 2007). In particular, interactions with AMF are thought to be important in invasive plant success through a number of mechanisms (Richardson et al. 2000; Pringle et al. 2009; Shah et al. 2009). These mechanisms include suppression of AMF by non-mycorrhizal invaders (Roberts and Anderson 2001; Stinson et al. 2006; Callaway et al. 2008), receipt of greater benefits from associations (Marler et al. 1999; Zabinski et al. 2002; Stampe and Daehler 2003; Carey et al. 2004; Harner et al. 2010), reduced dependence on AMF (Seifert et al. 2009; Vogelsang and Bever 2009), and alterations to the AMF community (Mummey and Rillig 2006; Niu et al. 2007; Vogelsang and Bever 2009; Shah et al. 2010; Zhang et al. 2010).

Cheatgrass (*Bromus tectorum* L.) is a non-native invasive winter annual grass that is responsible for severe alterations to the sagebrush steppe ecosystem of western North America, which was historically dominated by big sagebrush (*Artemisia tridentata* Nutt.) (D'Antonio and Vitousek 1992). Overgrazing of native perennial grasses by cattle allowed *B. tectorum* to gain a foothold *(*Klemmedson and Smith 1964; Knapp 1996). *B. tectorum* burns readily and has reduced the fire return interval substantially in invaded communities (D'Antonio and Vitousek 1992). Native species are not adapted to this

altered fire cycle and are subsequently replaced by B. tectorum (Klemmedson and Smith 1964; Young and Evans 1978).

B. tectorum is considered a facultative AMF host (Allen 1984). *B. tectorum* reduces AMF diversity colonizing roots of neighboring grass species (Hawkes et al. 2006), and soils invaded by *B. tectorum* have lower AMF density than uninvaded soils (Al-Qarawi 2002). However, the identity and diversity of AMF species associating with *B. tectorum* are not known. Alternatively, *A. tridentata* associates with many AMF (Allen et al. 1995). It is likely that *B. tectorum* invasion results in significant alteration to the AMF community, given the disparity that exists between *B. tectorum* and the historic native dominant, *A. tridentata*. The objective of this research is to compare AMF diversity associated with coexisting *B. tectorum* and *A. tridentata*. The hypothesis tested is 1) alpha diversity of AMF associated with *B. tectorum* is lower than alpha diversity associated with *A. tridentata*. Because of the difficulties in separating multiple influences on AMF, three distinct sites were identified where *B. tectorum* and *A. tridentata* are interspersed, to minimize the effects of site history and environmental heterogeneity.

Materials and Methods

Site Selection and Sample Collection

Three sites were identified in Colorado, Utah, and Wyoming, USA (hereafter referred to as the CO, UT, and WY sites, respectively) with dissimilar soil types, management histories, and plant community age (Table 1). The CO site was located in the Piceance Basin. Dominant vegetation included Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis* Beetle & Young), western wheatgrass (*Pascopyrum*

Table 1. Characteristics of *Bromus tectorum* and *Artemisia tridentata* sampling sites where roots and soils were obtained.

Site	Location (Lat., Long.)	Elevation	Soil Texture[1]	Soil Classification[1]	Landscape Position	Community Age
CO	39.9063°, -108.3970°	1990 m	loam	Fine-loamy, mixed Borollic Camborthids	basin floor	Mature sagebrush steppe
UT	40.4558°, -112.0484°	1950 m	cobbly sandy clay loam	Clayey-skeletal, smectitic, frigid Lithic Argixerolls	rocky, upland slope	Intermediate sagebrush steppe
WY	42.2557°, -104.7671°	1340 m	sandy loam	Coarse-loamy, mixed, superactive, calcareous, mesic Ustic Torrifluvents	bottom-land floodplain	Immature sagebrush steppe

[1]Soil series data were obtained using NRCS Web Soil Survey: http://websoilsurvey.nrcs.usda.gov/app/HomePage.htm

smithii (Rydb.) A. Löve), muttongrass (*Poa fendleriana* (Steud.) Vasey), *B. tectorum*, and *Alyssum* spp., based on visual cover estimates obtained at the time of sample collection (data not shown). The UT site was located on a mountain slope that is managed using controlled burns to reduce the dominance of woody vegetation. Dominant plant species included Gambel oak (*Quercus gambelii* Nutt.), bulbous bluegrass (*Poa bulbosa* L.), arrowleaf balsamroot (*Balsamorhiza sagittata* (Pursh) Nutt.), *Artemisia tridentata* Nutt. ssp. *wyomingensis*, *B. tectorum*, and antelope bitterbrush (*Purshia tridentata* (Pursh) DC). The WY site was located in a 10 ha grazing exclosure, where *A. tridentata* and other native vegetation was able to re-establish. Dominant vegetation included *B. tectorum*, needle and thread grass (*Hesperostipa comata* (Trin. & Rupr.) Barkworth), deathcamas (*Zigadenus venenosus* S. Watson), silver sagebrush (*Artemisia cana* Pursh), and *Artemisia tridentata* Nutt. ssp. *wyomingensis*.

Sixteen interspersed *B. tectorum* and *A. tridentata* individuals were identified at each site. By selecting coexisting populations with interspersed individuals, external

environmental influences were minimized. To appropriately compare an annual grass to a long-lived composite shrub, only *A. tridentata* juveniles less than 15 cm in height were selected, as juveniles were believed to be closer in age, C fixation capacity, height, mass, and root volume to *B. tectorum*. To minimize the potential effects of host species' effects on AMF associations of neighboring plants, individuals selected for sampling were at least 50 cm from individuals of the other host plant being sampled. Samples were collected from May 19-22, 2008. This period was selected to coincide with peak root colonization by AMF. For *A. tridentata*, this period was identified as occurring in late spring (Trent et al. 1994). For *B. tectorum*, this peak was not known, but was assumed to occur during a time period similar to other C_3 grasses, which is just before flowering in late spring (Bentivenga and Hetrick 1992). Samples were collected by excavating soil around each individual host to a diameter and depth of 15 cm. A subsample of roots was removed from each plant and a composite soil sample was removed from the excavated soil. Samples were placed in 50 ml conical centrifuge tubes and placed on ice for transport to the laboratory.

Molecular Analyses of Samples

Roots were rinsed in sterile, DNA-free water, and DNA was extracted using the FastDNA Spin Kit (MP Biomedicals, LOCATION). DNA was extracted from a maximum of 50 mg of roots, using the entire root subsample for *B. tectorum* and only fine roots for *A. tridentata*. For soils, DNA from 400 mg (dry weight) soil was extracted using the FastDNA Spin Kit for Soil (MP Biomedicals, Irvine, CA, USA). AMF DNA was amplified using the AM1 (5' – GTT TCC CGT AAG GCG CCG AA – 3') - NS31 (5' - TTG GAG GGC AAG TCT GGT GCC – 3') primer pair (Simon et al. 1992;

Helgason et al. 1998). PCR amplifications were conducted using the Expand High Fidelity Plus PCR System (Roche, LOCATION) with 25 uL reaction mixtures containing 2.5 uL of 2uM of each primer, 1 uL of 100 uM dNTPs, 1 uL Expand High Fidelity Enzyme Mix in 1.25 U/uL, 2.5 uL of 1mM MgCl2, 5 uL buffer, 1uL 1% BSA, 1 uL of 10 ng/uL template DNA, and 8.5 uL H2O. Reaction conditions were as follows: hot start, 94 °C for 2 minutes, 10 cycles of: 94 °C for 15s, 58 °C for 30s, 72 °C for 45s; 20 cycles of: 94 °C for 15s, 58 °C for 30s, 72 °C for 45s +5s per cycle; 72 °C for 5 minutes. PCR products were purified using the GENECLEAN Turbo Kit (Qbiogene, Montreal, QC, Canada), verified using agarose gel electrophoresis and quantified on a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

Positive PCR products were ligated into a pCR®2.1-TOPO vector using a TOPO TA cloning kit (Invitrogen, Carlsbad, CA, USA). Ligated products were transformed into One Shot® chemically competent *Escherichia coli* cells (Invitrogen). The transformed cells were spread onto a Luria-Bertani (LB) (Becton Dickinson, Franklin Lakes, NJ, USA) agar plate containing 50 µg/ml ampicillin and were incubated overnight at 37 °C. Fifteen well isolated ampicillin resistant colonies were selected randomly from each sample, transferred to 96-well plates and incubated for 24 hours using a shaker incubator at 37 ºC and 320 rpm in 2x LB broth containing 50µg/ml ampicillin. After incubation, the plates were centrifuged at 1500 x g for 7 minutes for further plasmid DNA isolation. Plasmid DNA isolation was performed using the Montage Plasmid Miniprep$_{HTS}$ Kit (Millipore, Billerica, MA, USA) following manufacturer's instructions. Isolated DNA was sequenced by the Colorado State University Proteomics and Metabolomics Facility using an ABI 3130xL Genetic Analyzer.

Editing, Alignment, and Matching of Sequence Data

Vectors were trimmed and raw sequences were edited using Geneious Pro version 5.0.4 (Biomatters Ltd., Auckland, NZ) with the following criteria: minimum sequence length of 300 bp and high quality DNA greater than 80%. Sequences resulting from editing were analyzed for chimeric sequences by the Bellerophon server (http://comp-bio.anu.edu.au/bellerophon/bellerophon.pl) using Huber-Hugenholtz correction and a window size of 200 bp (Huber et al. 2004). Sequences passing the above screening were assembled using custom assembly parameters: 50 bp word length with an index word length of 13 bp, maximum gap size of 1, 2% maximum gaps per read, and 2% maximum mismatches. Assembled sequences were then compared to the BLAST database to identify AMF sequences on 8 March 2011. All AMF sequences were submitted to GenBank under accession numbers JF683552-JF683578).

Phylogenetic Analyses of Sequence Data

AMF sequences meeting the above criteria were aligned using Geneious Pro version 5.0.4, a global alignment with free end gaps, eight refinement iterations and the following parameters: 65% similarity cost matrix, gap open penalty of 12, and gap extension penalty of 3. A neighbor-joining phylogenetic tree was constructed from the aligned sequences using the following parameters: consensus tree with a support threshold of 50%, Tamura-Nei genetic distance model, *Geosiphon pyriformis* as outgroup, and resampled by bootstrapping with 100 replicates.

Statistical Analyses of Sequence Data

AMF DNA sequences were analyzed by calculating diversity measures for the data. Diversity of AMF sequences was compared between hosts at each study site and

between hosts across study sites using t-tests with alpha = 0.05. Alpha diversity was calculated as the mean unique DNA sequences per sample group (root or soils samples associated with each host species at each site). Total number of unique sequences across a sample group represented gamma diversity. Beta diversity was calculated from alpha and gamma diversity, using the formula $\beta = (\gamma/\alpha)-1$ (Whittaker 1972). Site and host effects and their interaction on AMF sequence composition was analyzed for roots and soils separately using multivariate analyses of variance (MANOVA) with alpha = 0.1 in SAS version 9.1 (The SAS Institute, Cary, NC, USA). Composition of AMF sequences in roots and soils were compared separately between hosts at each site and across sites using multiple response permutation procedure (MRPP) with 4,999 permutations and alpha = 0.1 using PC-ORD version 5.31 (MjM Software, Gleneden Beach, OR, USA). To identify potential AMF associations with a particular host within and across sites, indicator species analysis was conducted using PC-ORD with 4,999 permutations for roots and soils with alpha = 0.1.

Results

DNA was extracted from 63 of 96 root and 82 of 96 soil samples. Failed samples were not randomly distributed, with 24 of the 33 failed root samples being sagebrush roots (11 of 16 sagebrush root samples from the UT site failed). Failed soil samples were also clustered, with 6 of the 14 samples occurring from sagebrush soils at the CO site. Of the samples yielding extractable DNA, one *A. tridentata* and six *B. tectorum* samples contained no AMF DNA, while six *A. tridentata* and eight *B. tectorum* soils contained no AMF DNA.

A total of 1612 sequences were assembled and aligned, resulting in identification of 27 unique AMF sequences (Figure 1). These sequences comprised 4 genera (*Diversispora*, *Glomus*, *Pacispora*, and *Scutellospora*) (Figure 1). Nine sequences clustered with a known species isolate. Naming convention for the sequences are

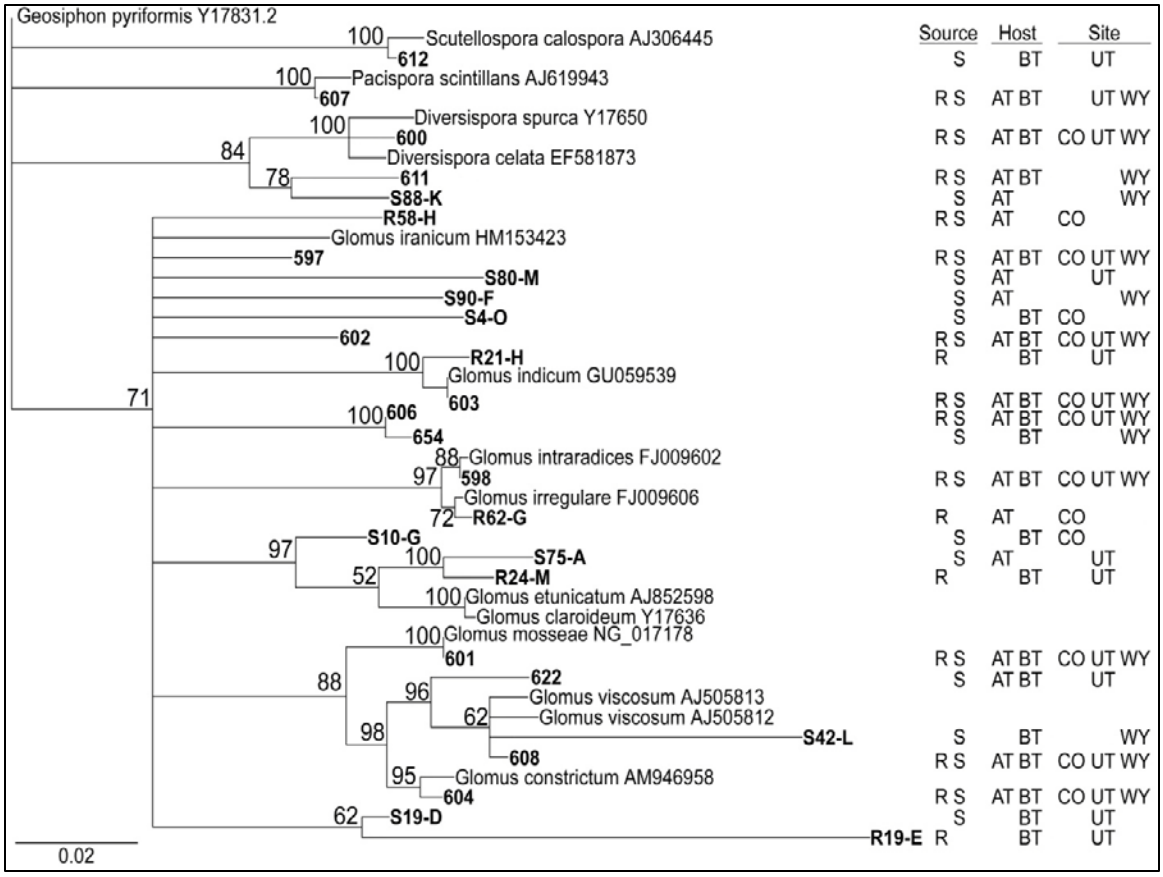

Figure 1. Neighbor-joining tree showing phylogeny of AMF DNA sequences isolated from *B. tectorum* and *A. tridentata* roots and soils. Isolated fungal sequences are in bold, identified sequences from GenBank are provided with species names and accession number. Naming convention for the sequences are numbered based on contig numbers assigned to aligned sequences by the software (for example contig 597), and sample identification numbers for unique sequences that did not align (for example R19-E). Numbers represent bootstrap percentages from 100 iterations. Isolated sequences are identified by sample source (R = root, S = soil), host plant (AT = *Artemisia tridentata* (big sagebrush), BT = *Bromus tectorum* (cheatgrass)), and site (CO = Colorado, UT = Utah, WY = Wyoming) from where they originated, listed on the right side of the tree.

numbered based on contig (set of overlapping sequences) numbers assigned to aligned sequences by the software (for example contig 597), and sample identification numbers for unique sequences that did not align (for example R19-E). The most common sequence, 597, which occurred in root and soil samples from both hosts at all sites, did not cluster with a known isolate, but was most closely aligned with *Glomus iranicum* (Figure 1). Of the 27 AMF sequences identified, 16 occurred in root samples and 22 occurred in soil samples (Table 2, Figure 2). Five sequences were unique to roots, while 11 were unique to soils (Figure 2). The number of sequences isolated from each sample ranged from one to six for *B. tectorum* roots (mean 2.15), one to five for sagebrush roots (mean 2.17), one to five for *B. tectorum* soils (mean 1.41), and one to four for sagebrush soils (mean 1.61) (Tables 2,3). Across all sites, 13 AMF sequences were isolated from *B. tectorum* roots (three unique) and 11 AMF sequences were isolated from *A. tridentata* roots (two unique). The most common sequence, 597, occurred in 76% of root samples and 55% of soil samples (Figure 2). Nine sequences were common across all three study sites (597, 598, 600, 601, 602, 603, 604, 606, and 608) (Figure 1). One sequence (607) occurred at only two sites (UT and WY), and 17 sequences were unique to only one of the sites (Figure 1).

AMF diversity measures for both roots and soils are presented in Table 3. Diversity measures are as follows: α diversity is the species richness per sample, presented as mean richness of unique AMF sequences in replicate root or soil samples (host and site as groups); γ diversity is the total richness across sample units, presented as total richness of unique AMF DNA sequences across replicate samples (host and site as groups); and β diversity is the rate of change between replicate samples (i.e. number of

Table 2. Frequency of AMF sequence occurrence across samples by host plant and site for root and soil samples. Naming convention for the sequences are numbered based on contig numbers assigned to aligned sequences by the software (for example contig 597), and sample identification numbers for unique sequences that did not align (for example R19-E).

	Roots						Soils					
	Host						Host					
	B. tectorum			A. tridentata			*B. tectorum*			*A. tridentata*		
	Site						Site					
Sequence	CO	UT	WY	CO	UT	WY	CO	UT	WY	CO	UT	WY
	Frequency (%)											
597	42	71	85	82	100	100	29	93	31	20	80	63
598	33	36	69	64	90	50	36	7		10	13	13
600		21	54	9		25	7	21	23		27	38
601	17	14	38	9		13	21	7	8	10		13
602	8	7	38			12		29			27	
603		7				25	14	14			13	6
604			8	9			7	14		10	20	6
606			31	9		25					7	
607		7				13					13	
608	8		15						8		7	6
611			8									13
612								7				
622								7			7	
654									8			
R19-E		7										
R21-H		7										
R24-M		7										
R58-H				9								
R62-G				9								
S4-O							7					
S10-G							7					
S19-D								7				
S42-L									8			
S75-A											7	
S80-M											7	
S88-K												6
S90-F												6

Figure 2a. Root Samples

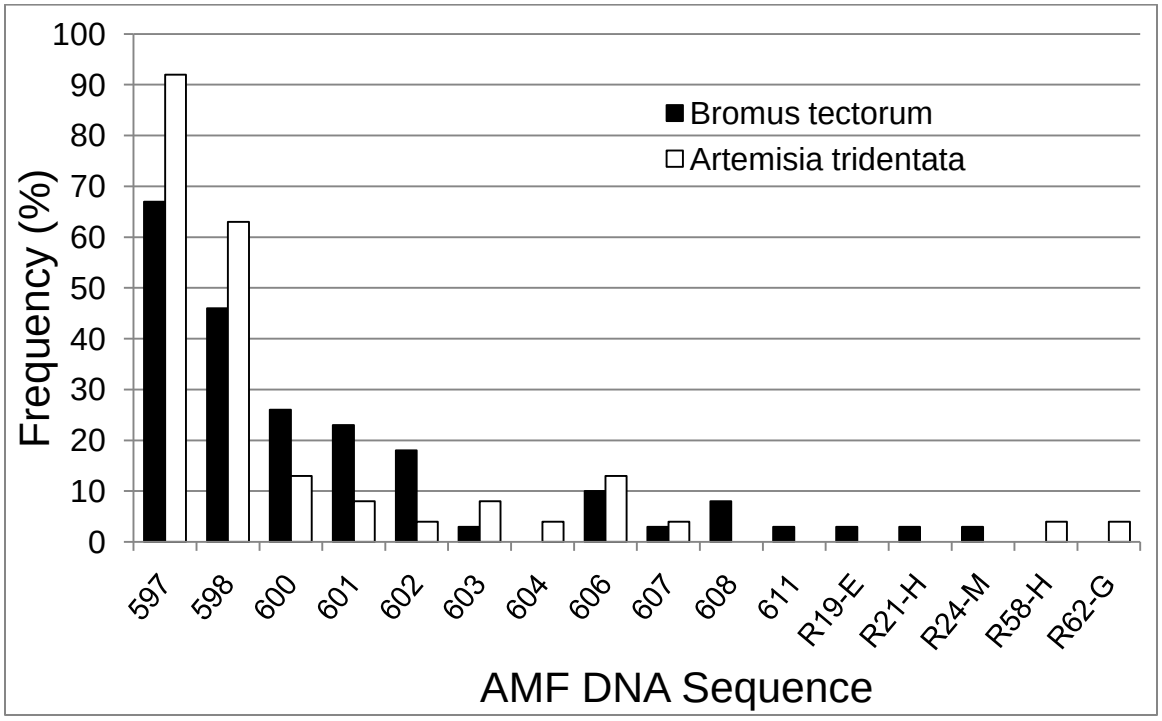

Figure 2b. Soil Samples

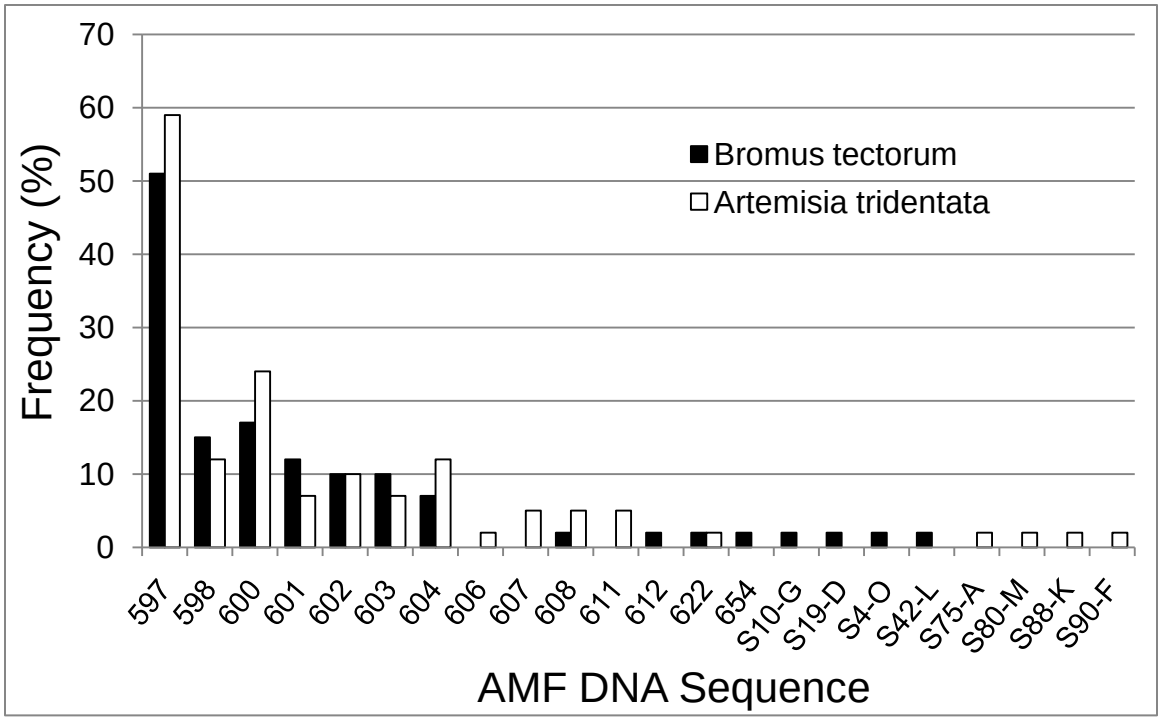

Figure 2. Frequency of AMF DNA sequences isolated from *B. tectorum* and *A. tridentata* roots and soils. Total frequency of AMF sequences across all sample sites, based on occurrence in either roots or soils from *B. tectorum* or *A. tridentata* host plants. Black bars indicate *B. tectorum* associations, white bars indicate *A. tridentata* associations. Naming convention for the sequences are numbered based on contig numbers assigned to aligned sequences by the software (for example contig 597), and sample identification numbers for unique sequences that did not align (for example R19-E).

a. Total frequency of AMF sequences isolated from *B. tectorum* and *A. tridentata* roots.

b. Total frequency of AMF sequences isolated from soils under *B. tectorum* and *A. tridentata*.

unique communities) in a group of replicate samples (host and site as groups), calculated here as $\beta = (\gamma/\alpha)-1$ (Whittaker 1972). Beta diversity represents the rate of change in community composition between samples in a group, i.e. the number of unique communities in a sample set. Alpha diversity of AMF DNA sequences did not differ between host plants for either root or soil samples across study sites (Table 4). However, alpha diversity between *B. tectorum* and *A. tridentata* soils at the WY site did differ (Table 4), as soils underneath *A. tridentata* contained almost twice as many mean AMF sequences as soils underneath *B. tectorum* at this site (Table 3). Roots and soils at the CO site differed in alpha diversity of AMF sequences at an alpha of 0.1 (Table 4), with *A. tridentata* roots and soils exhibiting greater diversity than B. tectorum (Table 3).

Site had a significant effect on composition of AMF communities, both in roots and soils (Table 5). Host roots differed significantly in their AMF composition across sites, while soils associated with the different host plants did not differ (Tables 5 and 6). AMF composition in roots was highly similar across host plants at the CO site, somewhat dissimilar at the WY site, and statistically different at the UT site (Table 6). All groups

Table 3. Diversity of AMF root and soil sequences within and across sites and plant hosts.

	Plant Host											
	B. tectorum				*A. tridentata*				Total			
		Diversity				Diversity				Diversity		
Root Samples												
Site	N[1]	α[2]	β[3]	γ[4]	N	α	β	γ	N	α	β	γ
CO[5]	12	1.1	3.63	5	11	2.0	2.00	4	23	1.5	5.58	10
UT	14	1.9	4.38	10	5	1.8	0.11	2	19	1.8	4.34	10
WY	13	3.5	1.60	9	8	2.6	2.04	5	21	3.1	2.50	11
Total	39	2.2	5.05	13	24	2.2	4.07	5	63	2.2	6.41	16
Soil Samples												
Site	N	α	β	γ	N	α	β	γ	N	α	β	γ
CO	14	1.3	5.20	8	10	0.5	7.00	4	24	1.0	7.33	8
UT	14	2.1	3.83	10	15	2.3	4.29	12	29	2.2	5.91	15
WY	13	0.9	6.06	6	16	1.7	4.92	10	29	1.3	8.16	12
Total	41	1.4	10.64	15	41	1.6	8.84	16	82	1.5	13.57	22

[1]N = number of samples per group.
[2]Alpha diversity, calculated as mean AMF sequence richness per sample.
[3]Beta diversity, calculated as $\beta = (\gamma/\alpha)-1$ *(Whittaker 1972).*
[4]Gamma diversity, calculated as total AMF species richness across samples.
[5]CO, UT and WY are the Colorado, Utah and Wyoming, USA, sites, respectively.

indicated strong heterogeneity between samples (Table 6). No AMF DNA sequences were strongly associated with one host over the other, and provided no significant indicator species (data not shown).

Discussion

The AMF alpha, beta and gamma diversity measures, particularly in the roots, did not indicate a consistent trend between sites. A diversity measure that was higher at one

Table 4. Alpha diversity comparison between AMF DNA sequences amplified from *B. tectorum* vs. *A. tridentata* roots and soils using t-tests (α=0.05). Alpha diversity was calculated as mean AMF sequence richness per sample.

	Root AMF Sequences			Soil AMF Sequences		
Site	df	t Value	Pr > |t|	df	t Value	Pr > |t|
CO[1]	21	1.99	0.06	22	-1.83	0.08
UT	17	0.19	0.79	27	0.80	0.43
WY	19	-1.50	0.15	27	3.11	< 0.01
Total	61	0.60	0.55	80	0.86	0.39

[1]CO, UT, and WY are the Colorado, Utah, and Wyoming, USA, sites, respectively.

Table 5. Two-way multivariate analysis of variance testing significance of site, host, and site x host interaction effects on AMF DNA sequences isolated from *B. tectorum* and *A. tridentata* roots and associated soils (alpha = 0.1).

Effect	Num df	Den df	Wilks' Lambda[1]	F-Value	Pr > F
Root Samples					
Site	32	80	0.37	1.58	0.05
Host	16	40	0.58	1.85	0.06
Site x Host	32	80	0.49	1.05	0.41
Soil Samples					
Site	44	110	0.29	2.12	< 0.01
Host	22	55	0.76	0.79	0.72
Site x Host	44	110	0.57	0.81	0.78

[1]A Wilk's Lambda close to zero indicates a strong positive relationship, close to 1 indicates a weak relationship.

site for a particular host plant was lower for that host at another site, or there was no difference at another site. This observation could be a reflection of differences in plant community age or site heterogeneity. Differences between sites due to environmental factors are important to *A. tridentata*, where AMF associations differed drastically

Table 6. Multiple response permutation procedure with 4,999 permutations for the effects of host identity on AMF DNA sequence composition from roots and soils from three sites between hosts *B. tectorum* and *A. tridentata*.

	Roots			Soils		
Site	T[1]	A[2]	P	T	A	P
CO[3]	0.968	-0.041	0.912	0.841	-0.045	0.802
UT	-1.727	0.055	0.065	0.741	-0.013	0.759
WY	-1.174	0.029	0.123	0.883	-0.020	0.847
Total	-1.841	0.017	0.056	0.896	-0.006	0.842

[1]Test Statistic. T describes degree of group separation. A T close to zero indicates little separation. A T far from zero indicates strong separation.
[2]Agreement Statistic. A describes within-group separation. When A = 1, all observations are identical. When A = 0, within-group heterogeneity equals what is expected due to chance.
[3]CO, UT and WY are the Colorado, Utah and Wyoming, USA, sites, respectively.

between numerous sites (Allen et al. 1995). However, the differences observed could be due to the non-random lack of DNA samples from the *A. tridentata* roots and soils.

Failure to extract DNA from sagebrush root and soil samples reduced the overall power of this study, and led to comparisons of unequal sample sizes for all statistical analyses. The large differences between *B. tectorum* and *A. tridentata* roots at the Utah site undoubtedly skewed the gamma and beta diversity calculations, at the very least. *A. tridentata* produces numerous phenolic compounds (Brown et al. 1975), and numerous terpenoid compounds are exuded from its roots (Jassbi et al. 2010). Secondary compounds such as these can contaminate DNA and interfere with DNA extraction (Friar 2005). Thus, the difficulty in extracting DNA from these samples, given the high

concentration in sagebrush samples, was most likely a result of secondary compounds present in the roots that were also exuded into the soil.

This study isolated the AMF occurring in roots of and soils beneath coexisting *B. tectorum* and *A. tridentata* from three disparate sites in the semi-arid steppe ecosystem of the western United States. We found that although the mean richness of AMF species associated with *B. tectorum* and *A. tridentata* roots and soils did not differ. However, the effect of host on the composition of AMF associates was significant, most likely due to half of the AMF species colonizing roots only occurring in one of the two host species. This finding indicates that *B. tectorum* associates with a different community of AMF than *A. tridentata* even when the two host plants coexist and are interspersed within a site. These changes have important consequences for persistence of *B. tectorum* and reestablishment of native species. Because *A. tridentata* is the historic dominant plant species on most of the North American landscape invaded by *B. tectorum* (Knapp 1996), a shift in AMF community composition due to *B. tectorum* invasion could reduce recruitment success of *A. tridentata* in *B. tectorum*-invaded soils. *B. tectorum*, a highly invasive introduced winter annual grass, has been shown to reduce AMF density in invaded soils (Al-Qarawi 2002) and reduce diversity of AMF colonizing neighboring grasses (Hawkes et al. 2006). Allen et al. (1992) hypothesized that *B. tectorum* dominance may select for weedy mycorrhizal fungi. Hawkes et al. (2006) sought AMF associates of *B. tectorum*, but only identified non-mycorrhizal fungi. Thus, our study is the first to successfully identify AMF associating with *B. tectorum* in its invaded North American range.

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Chapter 5. Early seral plant species' interactions with an arbuscular mycorrhizal fungi community are highly variable

Introduction

Interactions between AMF and their plant hosts are highly variable (Francis and Read 1995; Bever et al. 1996; Castelli and Casper 2003). Plant responses to AMF are extremely variable in a general sense (Wilson and Hartnett 1988; Hetrick et al. 1992; Francis and Read 1995; van der Heijden et al. 1998; Bever and Schultz 2002), and in terms of AMF species-specific interactions (Bever and Schultz 2002). Plant host identity has a strong influence on AMF response (Johnson et al. 1992; Sanders and Fitter 1992; Bever et al. 1996; Bever and Schultz 2002). These differences are due not only to abiotic factors and host/symbiont identity, but are also affected by neighboring plants (Hausmann and Hawkes 2009), age of host (Husband et al. 2002), legacy effects of community development (Hausmann and Hawkes 2010), etc. The end result is that plant-AMF associations exert significant influences on community structure, where AMF diversity influences plant diversity (van der Heijden et al. 1998; Klironomos et al. 2000; Vogelsang et al. 2006) and vice versa (Eom et al. 2000; Hausmann and Hawkes 2010).

Plant community assembly is also strongly influenced by AMF (Janos 1980; Allen 1984; van der Heijden et al. 1998; Vogelsang et el. 2006). The transition of post-disturbance plant communities from non-mycorrhizal to facultative to obligately

mycorrhizal plant species occurs as AMF increase over time (Reeves et al. 1979; Janos 1980; Allen 1984). In many terrestrial plant communities, development is closely tied to the AMF community (Reeves et al. 1979; Gange et al. 1993). Plant species considered late successional or climax community dominant have repeatedly exhibited high dependence on AMF associations (Lindsey 1984; Hetrick et al. 1988). Early- and mid-successional plants are thought to improve the AMF community, thus facilitating highly AMF-dependent later seral species (Renker et al. 2004). Early-seral plants interact minimally with AMF, although Pezzani et al. (2006) hypothesized that these interactions in particular are more complex. Those early and mid-successional species that have been investigated are only categorized as either mycorrhizal or non-mycorrhizal based on root colonization or, at best, how responsive they are to inoculation (Pendleton and Smith 1983; Wilson and Hartnett 1998).

Non-native invasive plant species are known to alter the arbuscular mycorrhizal fungi (AMF) community (Callaway et al. 2008; Vogelsang and Bever 2009). *Bromus tectorum* L. invasion is no exception, and can reduce AMF density in invaded soils (Al-Qawari 2002) and lower AMF species colonizing the roots of coexisting native grasses (Hawkes et al. 2006). *B. tectorum* has invaded millions of hectares of sagebrush (*Artemisia* spp.) shrublands in western North America, converting them into annual grass monocultures (Young and Clements 2009). This astounding conversion of shrubland has numerous ecological impacts, including loss of sage grouse (*Centrocercus urophasianus* (Bon.)) habitat.

B. tectorum has previously been found to be unresponsive to AMF (Allen 1984), *Artemisia tridentata* Nutt., the dominant native plant species in these shrublands,

possesses a high level of specificity for both colonization by and response to various AMF species (Lindsey 1984). Given the evidence supporting the degraded mutualist hypotheses associated with non-native plant invasions (Vogelsang and Bever 2009), soils with a history of dominance by invasive plants such as *B. tectorum* are likely to lose AMF species important for key historic interactions, because the invader neither benefits from nor provides benefits for AMF. Overcoming such alterations could be accomplished through improved knowledge and exploitation of plant-AMF interactions in invaded soils. Given the high degree of disparity between plant species interacting with AMF, it is likely that certain native species interact in ways that improve restoration of invaded soils by promoting favorable associations.

If weedy species are variable in their interactions with AMF, native weedy species may be potentially useful for restoring invaded soils if they can increase AMF needed by the late successional plants that historically replaced them. The only way to adequately identify these complex interactions is to combine both plant and fungal responses to the associations. Aside from a handful of studies that measured sporulation of AMF associated with different host plants (Johnson et al., 1992; Sanders and Fitter 1992; Bever et al. 1996; Bever and Schultz 2002), interactions between most host plant species and AMF have been determined through observations of plant responses only. Given that AMF are not always mutualists, and can range from mutualistic to parasitic (Francis and Read 1995), this lack of AMF response measurement fails to provide critical information on how both host and symbiont fare from interactions. Information regarding how AMF respond to hosts is critical for utilization of these interactions to restore vegetation dependent on AMF.

In North American rangelands, forbs contribute the highest species richness of all vegetation forms (Sims et al. 1978; Pokorny et al. 2004). Forb species are highly variable in their responses to AMF colonization (Hetrick et al. 1992; Wilson and Hartnett 1998). Thus, there exists the potential for numerous species to exist that perform important ecological functions, yet are overlooked, including interactions with AMF. To identify some of these species, the hypothesis that plant responses to AMF are not indicative of AMF responses to plant hosts was tested. Because plant host responses to AMF communities cannot be determined by observing the effects of each AMF species individually (Gustafson and Casper 2006), we used an AMF community that assembled naturally in a soil invaded by cheatgrass. First, plant biomass responses of 17 plant species to a community of AMF were determined using soil from a disturbed rangeland in Wyoming, USA. Second, AMF colonization of a bioassay host was used to measure AMF density resulting after association with each of the 17 host plant species. Our long-term objective is to identify early-seral native plant species that could be used in restoration plantings on degraded sagebrush steppe habitats in western North America. However, we expect that the approach outlined here would be generally applicable to restoration of any terrestrial plant communities where late-seral species are highly AMF dependent.

Materials and Methods

Study Site

A site typical of disturbed sagebrush grassland was selected in eastern Wyoming, USA, approximately 9 km NW of Hartville (42° 24’ 14” N, 104° 48’ 35” W). The site (1490 m elevation) was historically dominated by *A. tridentata*, but the native shrubland

community was removed with 2,4-Dichlorophenoxyacetic acid approximately 60 years ago and seeded with *Agropyron cristatum* (L.) Gaertn., an introduced perennial grass (D. Kafka 2009, Camp Guernsey, WY, personal communication). This site was subsequently invaded by *B. tectorum* at least a decade ago. These species, along with *Bromus arvensis* L., another non-native invasive annual grass, comprise most of the vegetative cover at the site, based on 600 basal cover measurements taken with a point frame (*A. cristatum* = 67.1% of total vegetative cover, *B. tectorum* = 5.8% and *B. arvensis* = 4.8%). Eight 18-L soil samples were collected to a depth of 20 cm from random locations at the 0.5-ha field site, composited and mixed. Half of the soil was autoclaved for 1 h at 121 ºC and 103 kPa on 2 consecutive days. The sterile (AMF-) and non-sterile (AMF+) soils were diluted 1:1 (v:v) with sterile sand and placed in sterile pots.

An additional composite soil sample was collected in a similar manner from the site later in the growing season to coincide with maximum sporulation for identification of the most common AMF species. AMF spores were isolated using sucrose density centrifugation and identified using spore wall and other taxonomic criteria, as detailed by the International Culture Collection of (Vesicular) Arbuscular Mycorrhizas.

Plant Species

Seeds of 17 sagebrush steppe plants (Table 1), comprised of 12 forbs with diverse taxonomic and physiological representation (including a non-mycorrhizal host, *Cleome serrulata* Pursh) (Pendleton and Smith 1983)), two native C_3 perennial grasses, a native perennial C_4 grass, the dominant site colonizer (*B. tectorum*, an introduced invasive

Table 1. Plant species used in this study and their attributes.

Species	Family	Habit	Lifespan	Strategy[a]	Root System	Seed Biomass (g)
Bromus tectorum L.[b]	Poaceae	C_3 grass	Annual	R	Fine	3.03
Elymus elymoides (Raf.) Swezey	Poaceae	C_3 grass	Perennial	C-R	Fine	2.36
Poa secunda J. Presl	Poaceae	C3 grass	Perennial	S-R	Fine	0.43
Aristida purpurea Nutt.	Poaceae	C_4 grass	Perennial	C-R	Coarse	1.75
Asclepias speciosa Torr.	Asclepiadaceae	forb	Perennial	C-R	Taproot	6.33
Ambrosia psilostachya DC.	Asteraceae	forb	Perennial	C-R	Coarse	4.78
Coreopsis tinctoria Nutt.	Asteraceae	forb	Annual	R	Taproot	0.14
Grindelia squarrosa (Pursh) Dunal	Asteraceae	forb	Perennial	C-S-R	Taproot	1.57
Machaeranthera tanacetifolia (Kunth) Nees	Asteraceae	forb	Annual	R	Taproot	1.11
Ratibida columnifera (Nutt.) Woot. and Standl.	Asteraceae	forb	Perennial	C-R	Taproot	0.52
Cleome serrulata Pursh	Capparaceae	forb	Annual	R	Coarse	7.09
Monarda fistulosa L.	Lamiaceae	forb	Perennial	C-R	Coarse	0.36
Linum lewisii Pursh	Linaceae	forb	Perennial	C-R	Taproot	1.54
Sphaeralcea coccinea (Nutt.) Rydb.	Malvaceae	forb	Perennial	S-R	Taproot	0.91
Oenothera caespitosa Nutt.	Onagraceae	forb	Perennial	S-R	Taproot	0.35
Oenothera pallida Lindl.	Onagraceae	forb	Perennial	S-R	Coarse	0.65
Artemisia tridentata Nutt.	Asteraceae	shrub	Perennial	C-S	Taproot	0.23

[a]Based on strategies described in Grime (2002): C-R=Competitive Ruderal; C-S=Stress-Tolerant Competitor; C-S-R=Competitive Stress-Tolerant Ruderal; R=Ruderal; S-R=Stress-Tolerant Ruderal.

annual grass), and the historic late-seral dominant (*A. tridentata*) were used in the plant relative responsiveness study. Species selected were those with commercial availability and widespread occurrence. All selected species occur on Camp Guernsey or in adjacent counties.

Plant Relative Responsiveness

After autoclaving field soil to obtain the AMF- soil treatment, the autoclaved soil was re-inoculated with non-AMF microbes by filtering 200 g non-sterile soil through a Whatman (GE Healthcare, Fairfield, CT, USA) #1 filter (11 µm pores) using 2 L sterile deionized water and adding 10 ml of this non-sterile soil filtrate to each pot (Gemma et al. 2002). Inoculated microbes were allowed to stabilize for 1 week before planting. An unseeded AMF+ soil treatment was included as a control (to compare density of AMF without a host to treatments with a host for the duration of the experiment) in the subsequent AMF colonization study. Seeds of each of the 17 plant species were surface sterilized in 10% sodium hypochlorite solution for 5 minutes and placed into five 983-ml Deepots™ (Stuewe and Sons, Tangent, OR, USA) for each of the AMF+ and AMF- soils. After germination, seedlings were thinned to one individual per pot and grown in a glasshouse at 20 ºC night-time and 24 ºC daytime temperatures, and supplemented with sodium vapor lights to maintain 16 hours of daylight. Pots were allowed to dry thoroughly before re-moistening to mimic field environmental conditions, and re-randomized weekly to minimize microclimate effects.

After 120 days of growth (Wilson and Hartnett 1988), shoot biomass was measured by drying shoots at 60 ºC for 72 hours. Relative responsiveness for each species was calculated using the equation: [(mean mycorrhizal plant dry mass) – (mean

non-mycorrhizal dry mass) / (mean mycorrhizal plant dry mass)] x 100 (Wilson and Hartnett 1988). Biomass differences between soil treatments for each plant species were analyzed using paired t-tests (n = 10) with an alpha of 0.05 and the Satterthwaite method for unequal variances. Data met the assumptions of the t-test analysis based on residuals plots, box plots and Wilks-Shapiro normality tests, and no data transformation was necessary.

Root Colonization by AMF

A small root subsample was removed from each plant in the AMF+ soil treatment after removal of shoots for the plant responsiveness study. Samples were washed, cleared in 2.5% KOH for 30 min. at 90 °C, rinsed, acidified in 1% HCl for 4 h., stained in acid glycerol containing 0.05% trypan blue for 30 minutes at 90 °C, and destained in acid glycerol 30 minutes at 90 °C (Koske and Gemma 1989). Roots were observed under 400X magnification and the presence of hyphae, vesicles, arbuscules, and auxiliary cells were determined using 100 root intersections per sample with a crosshair reticle (McGonigle et al. 1990). Additionally, three random replicate root samples from the five total replicates for each host treatment in the AMF- soil were stained and observed for colonization by AMF.

Host-Dependent AMF Density

Effects of the plant hosts on AMF density were measured using a variation of the mycorrhizal colonization potential bioassay (Moorman and Reeves 1979). This bioassay accounts for all propagules, including spores, extraradicle hyphae, and colonized root fragments that are all capable of subsequent colonization of hosts (Moorman and Reeves 1979), and has been used to compare AMF densities in trained soils (Vogelsang and

Bever 2009). To measure the response of the AMF community to individual host species, the remaining material from each Deepot was utilized as inoculum for a colonization bioassay with a promiscuous host. For each Deepot, the root system (except for the unseeded control) was chopped into 1-cm fragments, and the chopped roots and remaining pot soil were thoroughly homogenized by mixing the material from the five replicate pots for each host treatment. A 150 ml composited soil-root mixture for each plant host species treatment was placed into each of five 164 ml SC10 Conetainers™. *Sorghum bicolor* (L.) Moench ssp. *drummondii* (Nees ex Steud.) de Wet and Harlan seeds were surface sterilized by agitating in soapy water for 10 minutes, rinsed and agitated in 10% sodium hypochlorite solution for 5 minutes. Each soil-root mixture was seeded with surface-sterilized *S. bicolor* and grown for 30 days in a glasshouse using the growing conditions described above. After the 30 days of growth, the root systems were collected, cut into 1 cm fragments and placed into vials containing 70% ethanol for measurement of AMF colonization by clearing and staining roots in trypan blue solution (Koske and Gemma 1989; McGonigle et al. 1990).

An untrained control from the initial field-collected soil was compared to all the subsequent treatments to measure the effect of each host plant species on AMF density. Immediately after the initial collection and homogenization of field soil for the plant responsiveness study, a preliminary AMF density study was conducted in the same fashion to measure the initial AMF density in the field soil. This baseline in the initial untrained field soil was used as a reference to discern host plant effects on AMF density in the trained soils.

Data were analyzed by comparing mean total percent AMF colonization in each host treatment with the reference soil mean total percent colonization using analysis of variance ($n = 82$, $df = 18$, $F = 18.85$, $P < 0.0001$) and Dunnett's Method (controls Type 1 experimentwise error rate), which separates means by comparing all treatments with a specified control, with an alpha of 0.05. Residuals plots, box plots, Wilks-Shapiro normality tests, and Levene's homogeneity of variance test all confirmed that the assumptions of the analysis were met. Values from plant responsiveness and bioassay root colonization were multiplied to develop a comparable measure of interaction strength.

Results

All host plant species in the AMF- soil treatment lacked AMF colonization, with the exception of *Aristida purpurea* Nutt. (0.33% colonization), *A. tridentata* (0.33%), *Linum lewisii* Pursh (0.67%) and *Oenothera caespitosa* Nutt. (0.67%). Only hyphae were observed. All plant species growing in the AMF+ soil were colonized with structures indicative of AMF (Table 2). With the exceptions of *Poa secunda* J. Presl. and *C. serrulata*, percent colonization of roots ranged from 31% to 80%. No structures indicative of Gigasporaceae (auxiliary cells, inflated hyphae, inflated arbuscular trunks) were observed, and most observed structures were indicative of Glomeraceae (regularly shaped ovoid vesicles, highly branched arbuscules from thin trunk, thin patchy hyphae). Taxa identified from spores isolated from the study site include (in order of frequency): *Glomus aggregatum* N.C. Schenck and G.S. Sm., *G. constrictum* Trappe (*constrictus*), *G. mosseae* Gerd. and Trappe, *Paraglomus occultum* (C. Walker) J.B. Morton and D. Redecker, *G. claroideum* N. C. Schenck and G. S. Sm., *Entrophospora infrequents* (I.R.

Table 2. Percent colonization of host plant roots after 120 days growth in field soil.

Species	Vesicles	Arbuscules	Hyphae	Total Colonization
Cleome serrulata	0.0	0.0	0.4	0.4
Poa secunda	0.2	0.4	2.6	2.6
Grindelia squarrosa	0.3	4.8	29.8	29.8
Artemisia tridentata	0.6	5.0	31.4	31.4
Asclepias speciosa	0.8	0.6	31.6	31.6
Coreopsis tinctoria	0.2	9.0	32.6	32.8
Linum lewisii	0.4	20.0	33.2	33.6
Monarda fistulosa	1.2	8.6	34.6	34.6
Elymus elymoides	3.4	3.8	35.8	36.0
Bromus tectorum	1.6	23.8	39.6	39.8
Machaeranthera tanacetifolia	1.0	9.8	39.6	39.8
Ambrosia psilostachya	1.2	12.2	47.4	47.6
Aristida purpurea	5.8	15.0	50.2	50.2
Ratibida columnifera	1.6	16.4	53.0	53.6
Oenothera caespitosa	1.8	33.6	62.8	62.8
Sphaeralcea coccinea	8.2	20.2	64.4	64.4
Oenothera pallida	1.8	43.6	80.0	80.0

Hall) R.N. Ames and R.W. Schneid., *G. eberneum/Diversispora spurca*-like, *G. microaggregatum* Koske, Gemma and P.D. Olexia, and an *Acaulospor*a sp.

Plant responses to the AMF community were highly variable (Figure 1). AMF density was also highly variable (Figure 1), although AMF species-level differences could not be differentiated by the methodology used. Only the no-host control, the non-mycorrhizal host, and *P. secunda* (most negative relative plant biomass response to AMF) treatments yielded mean AMF densities lower than the initial field soil, which

yielded 13.6% colonization of bioassay roots (Figure 1). None of the host-trained treatments reduced AMF colonization below the initial level, but eight plant species had a positive effect on AMF density (Figure 1). AMF response to host species followed no discernible trend.

Of the 17 interactions resulting from the studies (Table 3), only three were mutualisms (plant +, AMF +), three were plant-positive commensalisms (plant +, AMF 0), two were AMF-positive commensalisms (plant 0, AMF +), three were plant-negative amensalisms (plant -, AMF 0), three were AMF-positive parasitisms (plant -, AMF +), and null effects (plant 0, AMF 0) were observed with the non-mycorrhizal host (*C. serrulata*), *B. tectorum* and *A. tridentata*. Although both *B. tectorum* and *A. tridentata*

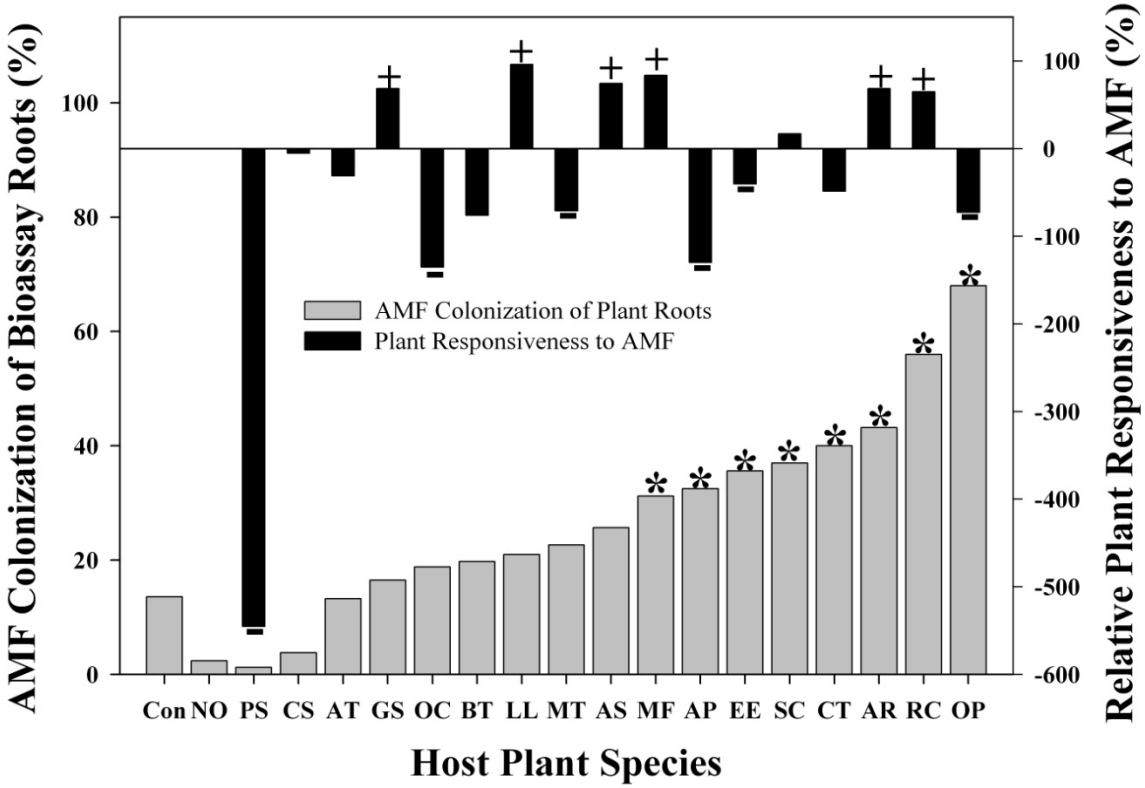

Figure 1 Plant responses to AMF are disparate from AMF responses to plant host identity. *Right y-axis*: Relative plant responsiveness calculated as [(mean mycorrhizal plant dry mass) – (mean non-mycorrhizal dry mass) / (mean mycorrhizal plant dry mass)] x 100. Biomass differences between soil treatments for each plant species were analyzed using paired t-tests (n = 10) with an alpha of 0.05. *Left y-axis*: AMF colonization measured by quantifying root colonization of a bioassay host (Sudangrass) grown in the trained soils containing AMF from each host plant species for 30 days. Data (n = 90) were analyzed by comparing mean total percent AMF colonization in each host treatment with the reference soil (Con) mean total percent colonization using analysis of variance (n = 82, df = 18, F = 18.85, P < 0.0001) and Dunnett's Method (mean separation that compares all treatments with a specified control) with an alpha of 0.05. *X-axis*: Abbreviations are as follows, with plant responsiveness t-test statistics in parenthesis: Con = initial field soil (control treatment for Dunnett's Method), NO = no plant host control, PS = *Poa secunda* (t = -4.80, P = 0.001), CS = *Cleome serrulata* (t = -0.22, P = 0.829), AT = *Artemisia tridentata* (t = -1.70, P = 0.128), GS = *Grindelia squarrosa* (t = 4.54, P = 0.016), OC = *Oenothera caespitosa* (t = -5.33, P = 0.004), BT = *Bromus tectorum* (t = -1.90, P = 0.123), LL = *Linum lewisii* (t = 11.52, P < 0.001), MT = *Machaeranthera tanacetifolia* (t = -4.59, P = 0.002), AS = *Asclepias speciosa* (t = 3.41, P = 0.022), MF = *Monarda fistulosa* (t = 7.55, P<0.001), AP = *Ambrosia psilostachya* (t = -5.50, P = 0.004), EE = *Elymus elymoides* (t = -2.57, P = 0.033), SC = *Sphaeralcea coccinea* (t = 0.64, P = 0.554), CT = *Coreopsis tinctoria* (t = -2.21, P = 0.058), AR = *Aristida purpurea* (t = -5.50, P = 0.013), RC = *Ratibida columnifera* (t = 2.71, P = 0.027), OP = *Oenothera pallida* (t = -6.07, P = 0.003).

+ Indicates species exhibiting a significant positive response to AMF.

- Indicates species exhibiting a significant negative response to AMF.

* Indicates host species that increased AMF colonization of bioassay roots significantly higher than initial soil (Con).

are considered mycorrhizal (Allen 1984; Lindsey 1984), neither species had a significant response to the AMF community, nor a significant effect on AMF density. Interaction strengths were calculated for each host plant (Table 3). Most of the calculations group into similar quantities for similar interaction types. The four hosts that did not group in this way also exhibited the greatest biomass variation in the plant responsiveness study, mostly due to bolting in a few individuals across treatments. If these four species are

removed, distinction of interactions observed becomes possible with this quantifiable index of interaction strength (mutualisms score highest, parasitisms score lowest, etc.).

Table 3. Types of interactions observed and calculated mycorrhizal interaction strengths.

Plant Host Species	Host Response[a]	AMF Response[b]	Interaction	Interaction Strength[c]
Ratibida columnifera	+	+	Mutualism	3.63
Aristida purpurea	+	+	Mutualism	2.96
Monarda fistulosa	+	+	Mutualism	2.60
Linum lewisii	+	0	Commensalism	2.02
Asclepias speciosa	+	0	Commensalism	1.91
Grindelia squarrosa	+	0	Commensalism	1.13
Sphaeralcea coccinea	0	+	Commensalism	0.63
Coreopsis tinctoria	0	+	Commensalism	-1.94[d]
Cleome serrulata	0	0	Null	-0.02
Artemisia tridentata	0	0	Null	-0.41
Bromus tectorum	0	0	Null	-1.50[d]
Poa secunda	-	0	Amensalism	-0.68
Machaeranthera tanacetifolia	-	0	Amensalism	-1.61[d]
Oenothera caespitosa	-	0	Amensalism	-2.54[d]
Elymus elymoides	-	+	Parasitism	-1.44
Oenothera pallida	-	+	Parasitism	-4.94
Ambrosia psilostachya	-	+	Parasitism	-4.22

[a]Based on plant relative biomass response to AMF.
[b]Based on AMF colonization of bioassay roots grown in host soil
[c]Calculated by multiplying percent relative mycorrhizal responsiveness of each host plant species by percent colonization of bioassay roots grown in soil trained by that host plant species, multiplied by 10.
[d]Species whose variation differences between treatments in the relative responsiveness study were highest (SD > 0.33, SE > 0.11)

Discussion

The wide variation in responses of host plant species to AMF observed in this study has been consistently shown for numerous plant species (Wilson and Hartnett 1988; Hetrick et al. 1992). The responses of the grasses to AMF were expected (positive response for the C_4 grass, negative or no response for C_3 grasses) (Wilson and Hartnett 1988; Hetrick et al. 1992). However, the degree to which *P. secunda* biomass was reduced when grown with AMF was surprising. The forb responses were not expected (Table 3), even based on previously proposed characteristics including phylogeny (Francis and Read 1995), seed size (Janos 1980) and root morphology (Hetrick et al. 1992) (Table 1). This wide variety of interactions between the AMF community and forb hosts is remarkable (Table 3), especially considering that in grasslands and shrublands the diversity of forbs far outweighs all other vegetation types (Sims et al. 1978; Pokorny et al. 2004). Plants observed to increase AMF colonization of bioassay roots included species that were positively affected by, negatively affected by and non-responsive to AMF. This indicates a high variation in interactions between plant species and AMF that does not follow a known pattern, and underscores the importance of discerning species-specific effects among host plants and soil AMF communities.

While many other soil microbes are important in plant communities and also exert feedbacks on plants, AMF effects on plants are direct, significant and common. Plant-soil microbe feedbacks have proven to be an important mechanism driving assembly of plant communities (Kardol et al. 2006, 2007). Although negative feedbacks have been shown to dominate early-seral communities while positive feedbacks dominate late-seral communities, it has been speculated that these different outcomes were due primarily to

pathogens and mutualists, respectively (Kardol et al. 2006, 2007). However, AMF have also exerted a strong influence on plant community assembly in early succession by promoting mycorrhizal plants (Janos 1980; Allen 1984; Gange et al. 1993). Given the previously demonstrated importance of AMF and the results presented in this study, it is highly likely that negative feedbacks observed in colonizers may be due not only to pathogens, but to parasitic interactions with AMF as well. When considering feedbacks with AMF and the abilities of these interactions to promote either coexistence or dominance of associated plant species (Bever and Schultz 2002), some of the interactions observed in the present study illustrate how plant-AMF feedbacks might facilitate advancement of the plant community beyond a community dominated by ruderals. If the AMF species that parasitized the ruderals *Ambrosia psilostachya* DC. and *Oenothera pallida* Lindl. were species that could provide a strong positive feedback on a neighboring plant species, thereby facilitating a competitive advantage in the neighboring plant species and possible replacement of the parasitized ruderal(s).

Previous research has also indicated a strong effect of host plant species identity on AMF species composition (Johnson et al. 1992; Sanders and Fitter; 1992, Bever et al. 1996; Bever and Schultz 2002; Hausmann and Hawkes 2009). The differences in AMF colonization of bioassay roots was most likely due to different AMF species responding to the different host plant species. However, because changes in AMF species were not measured, it is not known exactly which AMF species were most affected by which host plants, either negatively or positively. Future research to compare specific changes due to association with native plants and the feedbacks of these changes on both invaders and

desirable natives could prove useful for practical restoration applications targeting individual AMF species.

These findings have important implications for ecological restoration, and are particularly relevant for two of the host plant species used in this research, *B. tectorum* and *A. tridentata*, given the historical invasion replacement between these species. The degraded mutualist hypothesis (Vogelsang and Bever 2009) indicates that invasive plants such as *B. tectorum* are not highly responsive to AMF, and as poor hosts, are associated with an AMF reduction in invaded soils. Previous research indicates that *A. tridentata* can be highly responsive to AMF when associating with certain AMF species, but can also be non-responsive when associating with other AMF species (Lindsey 1984). In the present study, it is likely that because of the historic *B. tectorum* invasion, this mutualist degradation led to a reduction of AMF in the soil. This reduction may have caused AMF species most beneficial to *A. tridentata* to disappear or be severely diminished, which is perhaps why the results show no response by big sagebrush.

Based on the results of this study, it appears that host plant response to AMF is unrelated to AMF response to host plants. This is not unexpected, given that host species differentially affect the growth of AMF species (Johnson et al. 1992; Sanders and Fitter 1992; Bever et al. 1996; Bever and Schultz 2002; Hausmann and Hawkes 2009) and vice versa (Bever and Schultz 2002), and that different AMF species possess different growth strategies (Hart and Reader 2002). It also appears that invaded soils may be missing key microbial components necessary for establishment of desirable native vegetation. However, it is interesting that ruderal plant species, which are considered only facultative hosts of AMF, can interact in such diverse ways with the same AMF community.

Further, the diversity of interactions other than mutualistic between the host plants and a natural assemblage of AMF species was unexpected. Because of the possible feedbacks that result from these specific host-AMF interactions (Bever et al. 2002), as well as the effects of neighboring plants on host-specific AMF associates (Hausmann and Hawkes 2009), the diversity of interactions found between ruderal plant species and the AMF community could be important for both plant and AMF species recruitment, replacement, persistence and dominance. Knowledge of the specific interactions between native annuals and other early-seral plant species with AMF in degraded soils could improve restoration of degraded soils through subsequent facilitation of AMF-dependent later seral plant species.

Conclusions

The results of this study indicate that native early-seral plant species have highly variable interactions with an AMF community. Some early-seral plant species can facilitate rapid increases of AMF density in soils. These increases may be important for restoring soils where invasion by poor host plants or other disturbances have reduced AMF densities below levels that can support desirable vegetation.

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Chapter 6. Synthesis

Based on the results of research presented in this dissertation, cheatgrass appears to be a marginal host for AMF and alters the AMF community through a variety of mechanisms compared to big sagebrush. However, native plant species interact with AMF associated with cheatgrass in a variety of ways, and these interactions could be utilized to target the AMF community to aid in restoration of invaded lands.

Cheatgrass is not an ideal AMF host, as it never becomes highly colonized by AMF throughout its life. Further, because of cheatgrass' strategy as a winter annual and ability to suppress most other vegetation, the loss of a plant host through a large portion of the growing season could additionally influence the AMF community by reducing AMF species that are active during this period of inactivity by cheatgrass. Because AMF do not readily colonize cheatgrass and cheatgrass invasion renders highly invaded areas without an AMF host through a large portion of the growing season, cheatgrass invasion has the potential to profoundly influence the AMF community.

Based on DNA isolation from host roots, cheatgrass was colonized most frequently by AMF species most closely related to *Glomus iranicum* (which is possibly synonymous with *Glomus aggregatum* based on published descriptions), *Glomus intraradices*, *Diversispora spurca*, and *Glomus mosseae*. At the same three sites, big

sagebrush was colonized more frequently with AMF species most closely related to *Glomus iranicum* and *Glomus intraradices*, and less frequently with *Diversispora spurca* and *Glomus mosseae* compared to cheatgrass. Cheatgrass differs in composition of AMF species colonizing its roots compared to coexisting big sagebrush roots.

Based on culturing of AMF from roots and soils associated with hosts, the AMF species most frequently associated with both big sagebrush and cheatgrass are: *Glomus mosseae*, *Glomus aggregatum*, *Paraglomus occultum*, and *Glomus eberneum*. Two AMF species (*Archaeospora trappei* and *Glomus viscosum*) associated with big sagebrush more frequently than with cheatgrass. Big sagebrush individuals associate with more AMF species than cheatgrass, based on spore cultures, and the sagebrush-associated AMF communities are more similar from one individual host to the next compared to cheatgrass. Cheatgrass invasion has the potential to reduce AMF diversity within a site, alter distribution of the remaining AMF as individuals associate with fewer AMF species, and remove diversity hotspots compared to big sagebrush. These differences indicate that big sagebrush individuals associate with a greater number of AMF species than cheatgrass, that these diversity hotspots are more similar across a population of big sagebrush than cheatgrass, and some species associating with big sagebrush may be lost due to severe cheatgrass invasion. These alterations of the AMF community due to cheatgrass invasion may have lasting effects on the recovery and restoration of native plant species.

In comparing the DNA isolation method with the spore culturing method, several differences in the data were observed. First, while all 96 cultures yielded spores, many DNA samples did not yield extractable DNA (33 of 96 root samples, and 14 of 96 soil

samples). This lack of DNA was observed prior to PCR amplification and was not due to the absence of fungal DNA. Rather, because even pulverized roots did not yield usable plant DNA, this lack of DNA was likely the result of compounds present in the soils and roots that bound to the DNA, making it unsuitable for PCR. Big sagebrush contains numerous secondary compounds, many of which have been shown to bind to DNA. Given that 24 of the 33 unusable root samples were from big sagebrush, this indicates that big sagebrush root samples were more problematic than cheatgrass root samples. Unusable soil samples were evenly divided between cheatgrass and big sagebrush.

This lack of samples in the DNA study made statistical comparisons of diversity difficult, as few groups had equal sample sizes. This is especially important for gamma diversity, as more samples will usually result in greater diversity. Because beta diversity is calculated from gamma diversity, this measure is also difficult to apply to unequal sample sizes. This impairment is a likely explanation for why the DNA and culturing studies did not produce similar results. While the culture data exhibited a similar trend across sites and hosts, the DNA data did not.

The unequal sample sizes could partially explain why the indicator species identified in the culture data were not observed in the DNA data. However, one of these indicator species is not amplified by the primers used in the DNA study. This is another shortcoming of the DNA method: no primer set is available to amplify all AMF species. *Acaulospora delicata*, *Archaeospora trappei*, *Entrophospora infrequens*, *Paraglomus* occultum, and possibly some of the unidentified spores were not amplified by the primers.

While the DNA method has several shortcomings, this method is much faster at generating data than the culturing method. To obtain an adequate number of spores, 4 months of culturing is required. However, many AMF species require longer periods of growth to induce sporulation, necessitating multiple culturing cycles. Further, there is never a guarantee that all AMF species have sporulated, regardless of the number of culturing cycles used. Spores also require identification, which is extraordinarily difficult. Another shortcoming of the trap culture method is that collecting soil from underneath a target host plant can be influenced by many other factors than the current host. Neighboring vegetation can influence the AMF community, as well as previous plants growing in the vicinity of the sample space.

Given the benefits and drawbacks of both methods, a combined approach may be most useful in ecological studies such as those conducted in this dissertation. Culturing is time consuming, but allows identification of unknown species, overcomes selectivity in primers, and yields biological material that can be used for further studies of the AMF. DNA extraction is prone to inhibition and has imperfect primers, but is fast and allows higher levels of resolution such as within-root communities.

Native plant species' interactions with an AMF community influenced by cheatgrass, crested wheatgrass, and Japanese brome were highly variable. Some native ruderals were highly responsive to the AMF community, some were non-responsive, and some were negatively responsive. Conversely, AMF responses to the different hosts were also highly variable, and some native plant hosts provided large increases in AMF density during a short time period. These responses were not related, as some large increases in AMF density were associated with negative responses of the plant host to the

AMF, while positive host responses to AMF were associated with no changes in AMF density. The interactions of these native plant species with the AMF community could be useful for restoration of invaded sites, where highly responsive native plant species could be utilized as better competitors, while those plant species that have a significant positive influence on AMF density could be used to facilitate establishment of other native plant species that are more dependent on AMF.

While the research presented in this dissertation has provided information related to cheatgrass' interactions with AMF, more investigations are needed to develop useful applications for management of AMF communities. First, it is necessary to know the importance of the AMF indicator species associated with big sagebrush, as well as the other AMF species found to associate with cheatgrass and big sagebrush. This includes determining if some of these AMF species are more beneficial or detrimental to the respective hosts. AMF species found detrimental to cheatgrass and beneficial to big sagebrush would be the most useful for restoring big sagebrush in areas invaded by cheatgrass, should such species exist. Second, knowledge of interactions between host plant species and specific AMF species is necessary to facilitate real changes in the AMF community. Chapter 5 only measured AMF community-level responses to different host plants, but these responses are the sum of a number of species. It is likely that different responses between the hosts were due to different AMF species. Identifying these responses is essential to utilizing plants to affect the AMF community. Further, this study was only performed at 1 site. Knowing how the plant hosts differed between plant community types at 1 location, as well as between locations, could be vital information. Finally, research to combine all these components and expand to other sites and systems

will provide the greatest applications. For example, if *Glomus viscosum* is found to be highly beneficial to big sagebrush while being highly detrimental to cheatgrass, this knowledge carries little importance if *Glomus viscosum* is severely reduced in density or absent at a particular site. However, if an alternative plant is identified that is highly responsive to the AMF community at this particular site, while serving as the preferred host for *Glomus viscosum,* then promoting this plant species may be useful for restoring the AMF community to one that can best support big sagebrush establishment.

Because cheatgrass is a poor host for AMF and alters the AMF community, consideration for the AMF community must be incorporated into plant restoration endeavors at sites invaded by cheatgrass, especially those with long histories of cheatgrass dominance. Because cheatgrass does not associate with certain AMF species as frequently as big sagebrush, the loss of these species may hinder big sagebrush establishment. Identifying a reduction in these indicator species could prove useful for alleviating transitions from shrubland to annual grassland. Similarly, other AMF species reduced by cheatgrass may be needed by other native plant species. However, because native plant interactions with AMF are so variable, there exist certain native plant species that are responsive to AMF communities associated with cheatgrass invasion, and some native plant species that can rapidly increase AMF density in invaded soils. These interactions may be site-specific, so a focus on these interactions prior to restoration may improve success. Furthermore, detailed investigations of the importance of specific AMF to specific plant hosts and the role of alternative host plants in selective responses by AMF symbionts could provide the capacity to promote particular AMF species that are vital to recovery of invaded lands.