

CONSEQUENCE OF PRE-INOCULATION WITH NATIVE
ARBUSCULAR MYCORRHIZAE ON ROOT COLONIZATION AND SURVIVAL OF
WYOMING BIG SAGEBRUSH (*Artemisia tridentata* ssp. *wyomingensis*) SEEDLINGS
AFTER TRANSPLANTING

by

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A thesis

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DEDICATION

This body of work is dedicated to my family and predecessors, both human and otherwise, who made this moment possible and who inspire and enrich the next.

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ABSTRACT

Inoculation of seedlings with arbuscular mycorrhizal fungi (AMF) is a common practice aimed at improving seedling establishment. The success of this practice largely depends on the ability of the inoculum to multiply and colonize the growing root system after transplanting. These events were investigated in *Artemisia tridentata* ssp. *wyomingensis* (Wyoming big sagebrush) seedlings inoculated with native AMF. Seedlings were first grown in a greenhouse in sterilized soil (non-inoculated seedlings) or soil containing a mixture of native mycorrhizae (inoculated seedlings). Three-month old seedlings were transplanted to 24 L pots containing soil from a sagebrush habitat (mesocosm experiments) or to a recently burned sagebrush habitat (field experiments). The mesocosm experiments were started in the spring and fall of 2011 and seedlings were grown under natural climatic conditions. Field experiments, conducted within the Morley Nelson Snake River Birds of Prey National Conservation Area, were initiated in the spring and fall of 2012. At the time of transplanting the percent root colonization was negligible for non-inoculated seedlings and ranged from 24 to 81% for the inoculated seedlings, depending on the experiment. In most experiments, 5 or 8 months after transplanting colonization was about twofold higher in inoculated than non-inoculated seedlings. During the mesocosm experiments, inoculation increased survival during the summer by 25%. In the field experiments, increases in AMF colonization were linked to increases in survival during both cold and dry periods and one year after transplanting

survival of inoculated seedlings was 27% higher than that of non-inoculated ones. To characterize the effect of inoculation on the AMF community, DNA was extracted from the roots and amplified with AMF specific primers. The AMF taxa were characterized based on sequences from the LSU-D2 rDNA region. A total of 6 phylotypes were identified, two within the Claroideoglomeraceae and four within the Glomeraceae. In addition, sequences were grouped into operational taxonomic units (OTUs) with sequence similarities greater than 94%. This resulted in the identification of 29 OTUs. Ordination analyses, using non-parametric multidimensional scaling, indicated that inoculation did not alter the structure of the AMF community. Similarly, no differences in OTU composition were detected between seedlings harvested in different seasons. Individual seedlings, regardless of inoculation treatment, were simultaneously colonized by AMF belonging to 3 to 6 OTUs. The lack of significant differences in AMF communities among inoculation treatments and seasons appeared to have been related to the predominance of certain OTUs. In all experiments and treatments, four OTUs were dominant. Overall, the results indicate that the inoculum derived from native AMF contributed to the colonization of roots that developed after transplanting, resulting in higher levels of colonization than those naturally occurring in the soil without altering the AMF community. Furthermore, increases in root colonization were associated with increases in Wyoming big sagebrush seedling survival.

TABLE OF CONTENTS

DEDICATION	iv
ACKNOWLEDGEMENTS	v
ABSTRACT	viii
LIST OF TABLES	xii
LIST OF FIGURES	xiii
LIST OF ABBREVIATIONS	xvii
INTRODUCTION	1
MATERIALS AND METHODS.....	15
Plant and Fungal Material	15
Mesocosm Experiments	16
Field experiments.....	18
Growth measurements	19
Chlorophyll <i>a</i> fluorescence measurements	20
Gas exchange measurements	21
Carbon isotope discrimination	22
AMF Colonization	23
DNA isolation, amplification, cloning, and sequencing	23
Phylogenetic Analyses	25
Data analyses	26
RESULTS	29

Mesocosm Experiments	29
Transplanting in spring 2011	29
Transplanting in Fall 2011	33
Comparison between spring and fall transplanting.....	34
Field Experiments	35
Spring transplanting	36
Fall transplanting	37
Phylogenetic Analyses	39
AMF community composition in non-inoculated and inoculated seedlings	40
DISCUSSION	43
Pre-inoculation effects on mycorrhizal colonization after transplanting.....	43
Increased levels of colonization associated with increased seedling survival.....	51
Effect of native-derived inoculum on the AMF community present in the seedlings.....	56
Conclusions.....	63
Literature cited	65
APPENDIX A	83
Tables	83
APPENDIX B	89
Figures.....	89

LIST OF TABLES

Table 1.	Total biomass and shoot over root ratio of inoculated and non-inoculated sagebrush seedlings transplanted on May 6, 2011. Mean ($\pm$ SE) of three to five replications. Means not labeled by the same letter are statistically significant according to the Tukey HSD test ($p < 0.05$) 86
Table 2.	Test for significance of time, treatment, and time x treatment on chlorophyll fluorescence and gas exchange parameters of sagebrush seedlings in the spring mesocosm experiment..... 87
Table 3.	Total biomass, shoot to root ratio, and foliar carbon isotope ratios ($\delta^{13}\text{C}$) of non-inoculated and inoculated sagebrush seedlings transplanted on October 1, 2011. Mean ($\pm$ SE) of 8 seedlings measured seven and half months after transplanting. Within a column, means labeled by the same letter are not statistically significant.....88
Table 4.	Percent total mycorrhizal and arbuscular colonization at the time of field transplanting in spring and fall 2012. Mean ($\pm$ SE) of 4 to 6 seedlings. Within a column, means labeled with different letters are significantly different ($p < 0.05$) based on Tukey-Kramer least square means test.....89
Table 5:	Richness (S) and Shannon-Wiener Diversity (H') indices of AMF OTU's isolated from the roots of (n) individual sagebrush seedlings (Mean $\pm$ SE) for each study. Within a particular experiment, values that differ significantly from the non-inoculated seedlings are indicated by an asterisk. For the spring 2012 experiment, sequences were also analyzed just before transplanting, pre-transplanted treatment. Total Diversity represents the diversity of pooled samples.....90

LIST OF FIGURES

- Figure 1. Environmental conditions that *Artemisia tridentata* seedlings experienced from May 6 to October 15, 2011. A, Soil volumetric water content (volume of water to volume of soil). B, Daily precipitation (mm). C, Daily average, minimum, and maximum temperatures (°C). 90
- Figure 2. Mycorrhizal colonization (A), survival (B), and shoot growth (C) of sagebrush seedlings growing in pots at the Idaho Botanical Gardens. A, Mycorrhizal colonization at the time of transplanting (May 6) and two and five months afterwards. Each bar represents the mean ($\pm$ SE) of at least five seedlings. For a particular date, bars marked by an asterisk are significantly higher than the non-inoculated seedlings ($p < 0.05$) based on an independent t -test. B, Fraction of surviving seedlings over the course of the experiment; different letters indicate significant differences ($p < 0.05$) based on a log-rank test. C, Shoot volume, each symbol represents the mean ($\pm$ SE) of all living seedlings in a particular treatment. The arrow, on July 1, indicates the initiation of irrigation for the watered treatment. 91
- Figure 3. Time course of ephemeral leaf senescence as measured by the fraction of plants with ephemeral leaves. Based on a log-rank test, drop of ephemeral leaves occurred later in well-watered plants than those experiencing natural precipitation ($p < 0.001$). 92
- Figure 4. Chlorophyll fluorescence parameters of *Artemisia tridentata* seedlings grown outdoors during the spring and summer of 2011. Non-photochemical quenching (A) and steady state fluorescence normalized to its dark-adapted state (F_s/F_o) (B) of well watered seedlings and of non-inoculated and inoculated seedlings receiving only natural precipitation. Each bar represents the mean ($\pm$ SE) of 35 measurements. Measurements were made biweekly from 6/29/11 to 9/22/11 and 5 measurements were conducted for each measuring date and treatment. Means not labelled with the same letter are significantly different ($p < 0.05$) based on Tukey-Kramer least square mean test. 93
- Figure 5. Influence of watering and mycorrhizal inoculation on gas exchange parameters of *Artemisia tridentata* seedlings grown outdoors during the spring and summer of 2011. Net photosynthesis (A), stomatal conductance (B), and photosynthetic water use efficiency (C) of well watered seedlings and of non-inoculated and inoculated seedlings that only received natural

	precipitation. Each bar represents the mean (+SE) of 35 measurements; measurements were made biweekly from 6/29/11 to 9/22/11 and 5 measurements were conducted for each measuring date and treatment. Means not labelled with the same letter are significantly different ($p < 0.05$) based on Tukey-Kramer least square mean test.	94
Figure 6.	Foliar carbon isotope ratios ($\delta^{13}\text{C}$) of <i>Artemisia tridentata</i> seedlings. Each bar represents the mean (-SE) of 5 seedlings. Leaves for $\delta^{13}\text{C}$ analysis were collected on October 6, 2011, at the end of spring transplanting experiment. Means not labelled with the same letter are significantly different ($p < 0.05$) based on Tukey-Kramer least square mean test.	95
Figure 7.	Weather conditions from October 1, 2011 to mid- June, 2012 at the Idaho Botanical Gardens experienced by seedlings in the fall mesocosm experiment. A, daily precipitation; B, Daily average, minimum, and maximum temperatures.....	96
Figure 8.	Mycorrhizal colonization (A), survival (B), and shoot growth (C) of sagebrush seedlings growing in pots at the Idaho Botanical Gardens from October 2011 to June 2012. A, Mycorrhizal colonization at the time of transplanting and seven and half months later. Each bar represents the mean ($\pm$ SE) of 8 seedlings. For a particular date, bars marked by an asterisk are significantly higher than the non-inoculated seedlings ($p < 0.05$) based on an independent t -test. B, Fraction of surviving seedlings over the course of the experiment. C, Shoot volume, each symbol represents the mean ($\pm$ SE) of all living seedlings in a particular treatment measured one month after transplanting (November 3, 2011), at the height of vegetative growth (June 8, 2011), and after the senescence of ephemeral leaves (July 12, 2012). Inoculation did not significantly affect seedling survival or shoot volume.....	97
Figure 9.	Weather conditions from June 1, 2012 to November 1, 2013 at the field site near Big Foot Butte, Snake River Birds of Prey, NCA. Panel (A) presents naturally occurring precipitation (mm) over the duration of the spring and fall 2012 field experiments. Panel (B): Temperature ($^{\circ}\text{C}$) is depicted showing daily average, minimum, and maximum values.	98
Figure 10.	Mycorrhizal colonization by treatment (A) and rooting depth (B), and survival (C) of sagebrush seedlings transplanted into the Big Foot Butte site on April 2012. Each bar represents the mean ($\pm$ SE) of six seedlings. In A, bars marked by an asterisk are significantly different from the non-inoculated seedlings ($p < 0.05$). In B, shallow roots were collected within the top 20 cm of the soil and deep roots below 30 cm; bars marked by an asterisk are significantly different from the shallow roots. C, Proportion of live seedlings over the course of the experiment. Based on a log-rank test,	

	survival was similar among treatments; x-axis in C indicates dates when survival was measured.	99
Figure 11.	Mycorrhizal colonization (A) and survival (B) of sagebrush seedlings transplanted into the Big Foot Butte site on October 4, 2012. In A, each bar represents the mean ($\pm$ SE) of six seedlings; bars marked by an asterisk are significantly different from the non-inoculated seedlings ($p < 0.05$). B, Proportion of live seedlings over the course of the experiment; different letters indicate significant differences based on a log-rank test.	100
Figure 12.	Phylogenetic tree of representative Glomeromycota LSU-D2 rDNA sequences detected in this study with the FLR3/FLR4 primers and database sequences of known Glomeromycota. Maximum Parsimony analysis with bootstrap support values above clade branches. Names and abundance of the six phylotypes identified are indicated on the right margin.	101
Figure 13.	Comparison of arbuscular mycorrhizae communities of non-inoculated and inoculated seedlings by nonmetric multidimensional scaling. Samples of <i>Artemisia tridentata</i> seedlings were collected several months after transplanting in the spring 11 (A) and fall 11 (B) mesocosm experiments, and the spring 12 (C), and fall 12 (D) field experiments. OTUs are represented by open triangles and individual seedlings with filled circles. The centroids for the each treatment are labeled (NI, non-inoculated seedlings; I, inoculated seedlings) and linked to the samples that relate to them. Ellipses represent 95% confident limits around the centroids of each variable. OTUs are labeled based on their phylotype (CI, <i>Claroideoglomus I</i> ; CII, <i>Claroideoglomus II</i> ; F, <i>Funneliformis</i> ; GI, <i>Glomus I</i> ; GII, <i>Glomus II</i> ; R, <i>Rhizophagus</i>) and OTU number within its phylotype.	102
Figure 14.	Analysis of arbuscular mycorrhizae communities by nonmetric multidimensional scaling. A, Comparison of AMF communities obtained from greenhouse-inoculated seedlings prior to transplanting (G) with those obtained from non-inoculated (NI) and inoculated (I) seedlings three months after transplanting in the 2012 mesocosm experiment. B, Analysis of AMF communities obtained from shallow (S) and deep (D) roots in the spring 2012 field experiment. C, Comparison of AMF communities in seedlings collected 5 and 8 months after the spring and fall 2011 mesocosm experiments, respectively. D, Comparison of AMF communities in seedlings collected 4 and 8 months after the spring and fall 2012 field experiments, respectively. OTUs are represented by open triangles and individual seedlings with filled circles. The centroids for each categorical variable are labeled and linked to the samples that relate to them. Ellipses represent 95% confident limits around the centroids of each variable.	103

Figure 15. Frequency of different AMF OTUs found to be colonizing roots of *Artemisia tridentata* seedlings. Results from the spring 11 (A), fall 11 (B) and spring 12 (E) mesocosm experiments, and from the spring 12 (C) and fall 12 (D) field experiments. F, Overall frequencies observed after combining the results from all the seedlings analyzed. In A to E, non-inoculated and inoculated seedlings were collected several months after transplanting. In E, greenhouse seedlings refer to inoculated seedlings that were analyzed just prior to transplanting. 104

LIST OF ABBREVIATIONS

AMF	Arbuscular Mycorrhizal Fungi
VWC	Volumetric Water Content
P	Phosphorus
N	Nitrogen
Zn	Zinc
MGR	Mycorrhizal Growth Response
F_0	Minimal Fluorescence
F_m	Maximal Fluorescence of dark adapted leaves
F'_m	Maximal Fluorescence following light exposure
F'_s	Steady-state Fluorescence
PSII	Photosystem II
NPQ	Non-Photochemical Quenching
CBE	Chlorazol black E
OTUs	Operational Taxonomic Unit (sequence homology >94%)
NMDS	Non-metric Multidimensional Scaling
NCA	National Conservation Area
S	Species Richness Index
$\delta^{13}\text{C}$	Ratio of Carbon isotope discrimination

INTRODUCTION

The soil microbial community plays important roles in terrestrial ecosystem processes through various interactions with other organisms (Loreau et al. 2001; Van der Putten et al. 2001; Wardle 2002). Microorganisms can affect the plant community via resource competition (Nordin et al. 2004), pathogenicity (Augsburger 1984), and by influencing the availability (Drigo et al. 2010) and translocation of nutrients (Smith and Read 1997) and water (Augé 2001). Furthermore, microorganisms can alter the composition of plant communities by differentially promoting or reducing plant productivity and fitness (Klironomos 2002; Pivato et al. 2007; Rout and Callaway 2012; Van Der Heijden et al. 2006; Van der Putten et al. 1993).

The arbuscular mycorrhizal fungi (AMF) are ubiquitous components of soil microbial communities. These fungi belong to the Phylum Glomeromycota (Schüßler et al. 2001) and form endosymbiotic associations with as many as 80% of terrestrial plants (Helgason and Fitter 2009; Klironomos et al. 1998; Sanders et al. 1996). Fossilized fungal hyphae and spores resembling those of arbuscular mycorrhizae have been discovered in sediments from the early Devonian and Ordovician era. These fossils are about 450 million years old, which coincides with estimates of the origin of the AMF lineage, based on molecular phylogenies (Corradi and Bonfante 2012; Redecker et al. 2000; Taylor et al. 1995). The long evolutionary history of the AMF-plant symbiosis suggests that AMF facilitated plant colonization of terrestrial habitats during the

Paleozoic era and have continued to influence plant and soil ecology since (Redecker et al. 2000; Taylor et al. 1995).

Arbuscular mycorrhizae are not known to reproduce sexually, but they propagate through the formation of multinucleate spores (Morton et al. 2004). In addition, vesicles and hyphal segments can give rise to an extensive network of heterokaryotic and coenocytic hyphae that extend from within the roots of the host into the soil. AMF are obligate biotrophs that rely on the plant host for organic carbon (Finley 2008). Due to this dependence of the fungus on its host, the term “myco-rrhiza” translates literally from Greek to “fungus-roots” and thus refers to both the fungus itself as well as the symbiosis between plant host and fungal symbiont. Emanating from the host plant roots, the extra-radical hyphae functionally increase the root absorptive surface area facilitating the uptake of nutrients, particularly those with low diffusivity in the soil such as phosphorus (P) and Zinc (Zn) (Smith et al. 2011). Phosphorus is one of the two macronutrients most often limiting plant growth; by increasing P uptake, mycorrhizal associations can result in significant increases in plant biomass (Cavagnaro et al. 2003; Smith et al. 2011; Smith and Smith 2011). Other effects of AMF on plants have received less attention, but there is evidence that AMF can reduce oxidative damage and increase plant tolerance to heavy metals, soil pathogens, and cold (Augé 2001; Cavagnaro et al. 2003; Drew et al. 2003; El-Tohamy et al. 1999; Finlay 2008; Maherali and Klironomos 2007; Marulanda et al. 2003; Paradis et al. 1995; Vivas et al. 2003; Welc et al. 2010).

Various studies have also investigated the effect of AMF colonization on plant drought tolerance (Augé 2001; Jayne and Quigley 2014). Mycorrhizal colonization tends to increase plant growth under water deficits and improve plant water status (Jayne and

Quigley 2014). Multiple processes may contribute to these effects. As soil moisture declines, P becomes more limiting due to the lengthening of the pathway for water and nutrient movement to the root surface (Sardans et al. 2007; Tinker and Nye 2000). Under these conditions, the presence of AMF becomes more critical for P uptake (Augé 2001; Suriyagoda et al. 2014). Consistent with this notion, improved water status and growth of AMF-plants under drought have often been correlated with concurrent increases in P uptake (Augé 2001; Dixon et al. 1994; Johnson et al. 2001; Li et al. 2014; Porcel and Ruiz-Lozano 2004; Sánchez-Blanco et al. 2004). AMF can also contribute to increased plant drought tolerance by water uptake via the extra-radical hyphae. However, this idea remains controversial. Even though the ramified hyphal network may have a large surface area, the hyphal diameter is small limiting the area through which water can move to the plant (Allen 2007). Furthermore, the flow of water has been shown to be bidirectional. Using a double compartment pot system, Ruiz Lozano and Azcón (1995) showed that water can move from the fungal compartment to the plant. In contrast, Allen (2007) using dyes and Querejeta (2003) using labeled isotopes, presented evidence of the flow of water from the host to the fungal hyphae through the process of hydraulic redistribution such that labeled or dyed water available to deep plant roots was translocated and exuded by the hyphal tips in the dry and shallow soil. Overall, the contribution of the extra-radical hyphae to water uptake may depend on the density of hyphae and the soil drying patterns (Allen 2007; Augé et al. 2003). Mycorrhizae can also affect plant water relations via increases in root hydraulic conductivity, osmotic adjustment, and stomatal conductance (Augé et al. 2014; Barzana et al. 2012; Barzana et al. 2014; Wu et al. 2013). Increases in root hydraulic conductivity and osmotic

adjustment tend to facilitate water transport and uptake, while increases in stomatal conductance increase water loss. Notwithstanding this loss, the plant may benefit through higher photosynthetic rates and thus more carbon for root and hyphal growth (Navarro Garcia et al. 2011; Wu et al. 2013). Effects of AMF on plant water relations may also be much more indirect. Mycorrhizal hyphae and spores contain the glycoprotein glomalin (Driver et al. 2005) that accumulates in the soil and increases soil aggregate formation, which increases the water holding capacity of the soil (Wright and Upadhyaya 1998).

Despite the various mechanisms by which AMF can improve plant water status, the effects of AMF on increasing drought tolerance are not as pronounced and consistent as those on increasing nutrient uptake (Augé 2004). Variability of the effect may reflect differences in the levels of water stress and the type of drought. Through a larger root system and increases in stomatal conductance, AMF plants can deplete water resources more rapidly and to greater extents than non-AMF controls (Allen et al. 1981; Augé et al. 2007; Osonubi et al. 1992). For prolonged droughts or in situations that the plant does not have access to deep water sources, the faster rate of soil water depletion may expose the plants to severe water deficits sooner. Such events would negate the positive effects of AMF on drought tolerance that the plants may have experienced under the initially milder water stress conditions (Suriyagoda et al. 2014).

Even though the AMF symbiosis typically has been considered mutualistic, the effects of AMF on plants can be quite variable (Alguacil et al. 2011; Augé 2001). The carbohydrate contributions to the fungal symbiont can constitute as much as 20% of the host's photosynthates (Snellgrove et al. 1982; Wang et al. 1989; Wright et al. 1998) and this substantial cost may tend toward parasitism in situations where resources are not

limiting to the host. Indeed, the mycorrhizal growth response (MGR) is often used as an index of host response to colonization (Facelli et al. 2010; Johnson and Graham 2013; Li et al. 2008). The MGR compares the plant biomass of AMF plants with that of non-AMF plants. When AMF plants have larger biomass than non-AMF plants the symbiosis is mutualistic. In contrast, a parasitic symbiosis would be determined by growth depressions when AMF plants have lower biomass than non-AMF plants. However, growth alone may not be sufficient to determine whether the symbiosis is mutualistic or parasitic (Grace et al. 2009; Jones and Smith 2004; Smith et al. 2003).

Another factor that can affect the nature of the AMF symbiosis is the particular AMF and plant genotypes involved in the association (Kiers et al. 2011; Sanders 2003). Even though AMF have low host specificity, it is becoming increasingly evident that the host plant can exert some selectivity on the AMF taxa colonizing its roots (Busby et al. 2013; Li et al. 2010a; Vandenkoornhuyse et al. 2002). For example, Torrecillas et al (2012a) evaluated the AMF community diversity associated with six co-occurring herbaceous plant species in a semi-arid prairie. Their results indicate that each species harbors a unique AMF population such that 18 of the 36 AMF phylotypes identified were specific to only one plant host species. The community composition showed interesting trends associated with the taxonomic identity of the plants evaluated, which belonged to three separate families; three species belonged to *Poaceae*, one species to the *Primulaceae*, and two to the *Asteraceae*. While one AMF group were only found to associate with plants belonging to the *Asteraceae*, seven AMF groups were exclusive to plants belonging to the *Primulaceae*. On the other hand, no unique AMF groups were found to specifically colonize the three *Poaceae* species. The reasons for the development

of preferential associations are unclear, but it appears that both plants and AMF have mechanisms to reinforce the most beneficial associations. This was illustrated in *Medicago truncatula* Gaertn. plants colonized simultaneously by several AMF rewarded more carbon to the species that transferred more P to the host roots (Kiers et al. 2011). Similarly, the fungus transferred more P to roots that provided ample carbon in exchange. In natural environments, these types of reciprocal rewards would tend to reinforce mutualistic partnerships while reducing neutral or detrimental plant/AMF associations (Johnson 2010).

In addition to the apparent discrimination by the hosts, AMF distribution and activity can vary seasonally and with climatic changes. Arbuscular mycorrhizae colonization and abundance have been sampled in a wide variety of habitat types including grasslands, desert, and tropical deciduous forests (Allen et al. 1998; Allen 1983; Lingfei et al. 2005; Santos-González et al. 2007; Sánchez-castro et al. 2012). Dumbrell et al (2011), utilizing molecular pyrosequencing techniques, identified distinct AMF communities in a temperate grassland when comparing summer to winter sampling. In contrast, Santos-González et al (2007) did not observe any changes in the AMF community assemblage colonizing *Prunella vulgaris* and *Antennaria dioica* throughout the growing season in a grazed grassland. While no shifts in community composition were observed in the herbaceous species sampled, the extent of root colonization in *A. dioica* declined substantially in the fall while no significant changes were seen in *P. vulgaris*. Allen (1983) characterized the colonization of *Atriplex gardneri* from April through July in a cold desert with the onset of drought stress and found that colonization decreased from 28% to 3% of the root system with no apparent shift in the identity of

colonizing AMF. The seasonal reduction in colonization extent or shift in composition are rarely studied but are likely to coincide with changes in host primary production, phenology, and soil nutrients. The above studies emphasize the dynamic and variable nature of AMF. Ultimately, host preferences and habitat conditions may result in resident AMF communities that are better suited for the local environment and that provide more benefits to the vegetation than non-native AMF assemblages (Ji et al. 2010; Lekberg et al. 2007; Mummey et al. 2009; Torrecillas et al. 2012a).

In a particular habitat, AMF abundance and composition can also be affected by various types of disturbances. Moorman and Reeves (1979) utilized a generalist host (*Zea mays*) to evaluate the effect of vegetation removal on AMF colonization. *Zea mays* growing in soil from disturbed and undisturbed areas showed 2% and 77% colonization of the root system, respectively. This suggests that the disturbance considerably decreased the amount of AMF inoculum present in the soil. Changes in vegetation composition brought about by weed invasion can also cause a decrease in the abundance and/or diversity of mycorrhizae (Mummey and Rillig 2006; Pringle et al. 2009; Torpy et al. 1999). This decrease may be exacerbated by fires, which tend to reduce the mycorrhizal density at the soil surface (Pattinson et al. 1999; Vilarino and Arines 1991; Wicklow-Howard 1989). Furthermore, in semiarid and arid regions, mycorrhizal spore density in the soil can be relatively low and mycorrhizal colonization of roots may be largely mediated by a below-ground hyphal network that maintains infectivity (Pattinson et al. 1999). By killing the vegetation, frequent wildfires are likely to lessen the spread of these networks, reducing the inoculum potential of the soil (Dangi 2010; O'Dea 2007; Torpy et al. 1999).

One of the most disturbed habitats in western North America is the sagebrush steppe within the Great Basin region. This region covers approximately 450,000 km² and contains much of the area bounded by the Columbia Plateau, Cascade-Sierra Mountain range, the central Rocky Mountains, and the Colorado Plateau. The sagebrush-steppe habitat occupies much of this region and is characterized by a vegetative community composed of perennial grasses, forbs, biological soil crusts, and several subspecies of *Artemisia tridentata* Nutt (big sagebrush). Increasingly, Great Basin sagebrush-steppe habitats are declining in both function and expanse (Knick et al. 2003; Noss et al. 1995). The loss of this habitat is due to numerous factors including urban expansion, overgrazing, and the invasion of exotic annual grasses. Urban expansion has extirpated native communities and increased fragmentation and accessibility to the habitat (Belnap 2003; Gelbard and Belnap 2003; Leu et al. 2008). Overgrazing degrades habitat quality by trampling the biological soil crusts and collapsing soil pores which decreases water infiltration and promotes surface runoff and erosion (Muschka and Hild 2006; Olff and Ritchie 1998; Walker et al. 1981). Invasive annual grasses, in particular *Bromus tectorum* (cheatgrass), have disproportionately influenced sagebrush communities by changing the historic fire regime (Whisenant 1990). This regime averaged one fire every 60 to 100 years where the fires burned over small areas at low temperatures due to the lack of fuel continuity in the interspaces between shrubs and grasses (Brooks et al. 2004; Whisenant 1990; Zouhar 2003). In invaded communities, where *B. tectorum* is abundant, highly flammable grass litter accumulates in the understory and interspaces providing a continuous fuel load that has increased fire frequencies to as much as one every 3 to 5 years (D'Antonio and Vitousek 1992). This fire frequency tends to exclude *A. tridentata*

and other fire intolerant components of the native vegetation. Furthermore, *B. tectorum* is able to rapidly recover after fires, successfully competing for resources with the native vegetation and perpetuating the shortened fire cycle (Pellant 1990). Ultimately, the altered fire regime leads to a drastic change in the plant community from one dominated by sagebrush, perennial bunch grasses, forbs, and biological soil crusts to one dominated by homogenous stands of exotic annual grasses (Anderson and Inouye 2001; Pellant 1990).

Intact native habitats have shown a higher resistance to exotic annual grass invasion. Consequently, rapid establishment of native vegetation following fires could reduce the abundance of invasive annuals and contribute to interrupt the grass invasion-fire cycle (Prev  y et al. 2010; Sheley 2010). Reseeding efforts with native perennial grasses have been relatively successful and this has decreased the frequency of exotic annuals in rehabilitated lands (Cox and Anderson 2004; Eiswerth and Scott Shonkwiler 2006; Evans and Young 1978). In contrast, reestablishment of other components of the vegetation, in particular *A. tridentata*, has proven more difficult. The predominant methods of direct re-seeding and seed broadcasting of *A. tridentata* have resulted in very low rates of establishment (Pellant et al. 2005). Even when high rates of seedling emergence have been achieved, particularly with drill seeding, subsequent seedling mortality led to very low shrub density (Cox and Anderson 2004; Monsen et al. 2004). Major factors responsible for low establishment include slow growth rates of sagebrush seedlings and summer drought (Dalzell 2004). Slow growth decreases the plant's ability to compete for resources and may prevent the seedlings from developing an adequate root

system to maintain hydration during the dry summer (Lambrecht et al. 2007; Stahl et al. 1998).

Artemisia tridentata is a dominant plant in the Great Basin and includes several subspecies that are generally non-overlapping due to variations in environmental requirements (Perryman et al. 2001; Rosentreter and Kelsey 1991). The presence of *A. tridentata* increases soil stability and contributes to the development of a heterogeneous landscape that facilitates the establishment of other native plants (Charley and West 1977; Davies et al. 2007; Ryel and Caldwell 1998). In addition, *A. tridentata* provides habitat and forage for local animals including obligate fauna such as greater sage-grouse (*Centrocercus urophasianus*), pygmy rabbit (*Brachylagus idahoensis*), and mule deer (*Odocoileus hemionus*) (Aldridge and Boyce 2007; Larrucea and Brussard 2008; Pederson and Welch 1982). Due to the contributions of *A. tridentata* to the structure and function of sagebrush habitats, there is considerable interest in reestablishing this species and with an emphasis on one of the subspecies, *Artemisia tridentata* ssp. *wyomingensis* Beetle & Young (Wyoming big sagebrush) (Pellant et al. 2005). This subspecies occurs throughout western North America where it usually grows in shallow soil and occupies xeric locations that receive 200-300 mm of annual precipitation (Perryman et al. 2001). The xeric environment and the shallow soil make reintroduction of Wyoming big sagebrush particularly difficult and this subspecies tends to be the most difficult to reestablish.

In natural habitats *A. tridentata* plants, including seedlings of ssp. *wyomingensis*, are often colonized by AMF (Busby et al. 2013; Carter et al. 2014; Trent et al. 1994a). The significance of this colonization for seedling establishment is, however, unclear. In a

greenhouse study, Stahl et al. (1998) demonstrated that Wyoming big sagebrush seedlings colonized by AMF survived to lower soil water potentials than non-AMF seedlings. A similar effect in the field could increase seedling survival during summer drought. Furthermore, in the field growth promotion by AMF could lead to a larger root system, enabling exploration of a greater soil volume, and overall to larger seedlings more tolerant of winter cold (Lambrecht et al. 2007). However, as discussed above, AMF colonization does not always cause growth promotion or an increase in plant stress tolerance. In the field in particular, variability in climatic and edaphic conditions may play a role in determining whether the symbiosis is mutualistic or parasitic (Hoeksema et al. 2010). Another complicating factor under field conditions is that seedlings are colonized to varying degrees by the resident AMF community present in the soil. Most studies on the effects of AMF on plant stress tolerance have been based on comparisons of non-AMF and AMF-plants, a situation that rarely occurs in the field. Consequently, under field conditions, the question is not only whether AMF affect seedling establishment, but also whether the natural levels of AMF inoculum in the soil are sufficient to optimize colonization and seedling survival.

In agricultural and horticultural systems the addition of mycorrhizal inoculum has resulted in increases in crop biomass or yield (Citernesi et al. 1998; Lekberg and Koide 2005; Norman et al. 1996; Wu et al. 2013). In barley, for instance, Al-Karaki and Clark (1999) determined that plant responses (dry mass and nutrient status) to increased colonization were related to the amount of AMF inoculum present. Native habitats have been less studied, however some results indicate that inoculation can also be beneficial for restoration of native habitats (Giri et al. 2004; Navarro Garcia et al. 2011; Ouahmane

et al. 2007; Stahl et al. 1988). In a Mediterranean ecosystem, Requena et al (2001) noted significant benefits following inoculation with native AMF and rhizobia; these additions increased AMF root colonization and plant establishment as well as soil fertility. In their experiment, some plants were also inoculated with non-native AMF; however, this inoculum did not persist in the soil (Requena et al. 2001). Differences in the persistence of non-native and native AMF inoculum were also reported by Weinbaum et al (1996). They conducted a reciprocal transplanting experiment where *Artemisia tridentata* ssp *tridentata* shrubs and AMF were moved from a site near San Diego, California to one near Reno, Nevada and vice versa. Over three growing seasons, AMF abundance remained stable when the inoculum was placed in its native site and with its native host population. In contrast, AMF abundance decreased when the AMF inoculum was placed at the non-native site with either *A. tridentata* population, or when it was placed in association with the non-native host population in its native soil. Taken together, these studies suggest that the origin of inoculum is an important factor in its ability to multiply and colonize plant root systems.

To test whether the AMF inoculum present in the soil is a limiting factor for colonization of Wyoming big sagebrush seedlings, in this study I conducted mesocosm and field experiments. These experiments involved transplanting non-inoculated seedlings and seedlings pre-inoculated with native AMF to pots filled with soil from a sagebrush habitat or to a recently burned sagebrush site. Several months after transplanting, I compared the natural levels of colonization with those of seedlings that have been pre-inoculated with AMF. The AMF inoculum was derived from soil collected at the same site where the soil was collected for the mesocosm experiments. The

production of the inoculum involved multiplication of the AMF originally present in the soil in pot cultures. This procedure can alter the AMF composition because the environmental conditions and hosts that are used for AMF multiplication are different from those in natural habitats (Sykorova et al. 2007). Furthermore in the field experiment, the inoculum was introduced into a new site, which could have led to differences in AMF composition between non-inoculated and inoculated seedlings. To assess the effect of inoculation on the richness, diversity, and composition of AMF taxa colonizing the roots of individual sagebrush seedlings, the D2 region of the 28S ribosomal RNA gene (LSU-D2) was amplified from root samples used to determine colonization.

A factor that may affect the ability of the inoculum to multiply and colonize the growing root system is the environmental conditions at the time of transplanting. Some support for this possibility comes from observations of seasonal variations in AMF colonization, which could be attributed to seasonal differences in AMF activity. Seasonal variations in colonization have been observed in mature shrubs of two subspecies of *A. tridentata*, *ssp. vaseyana* and *ssp. tridentata* (Trent et al. 1994a). For these shrubs (Trent et al. 1994a), AMF activity, as measured by hyphal length and arbuscular colonization, was positively correlated with soil moisture. In the Great Basin, transplanting of Wyoming big sagebrush has been conducted during spring or alternatively in early fall. At these times, the seedlings and AMF experience distinct edaphic and climatic conditions. Such differences may affect AMF growth after transplanting as well as AMF impact on seedling establishment. To investigate these possibilities, I conducted transplanting experiments in both spring and fall.

Overall, the aims of this study were to answer the following questions. Can the natural levels of AMF colonization be increased by pre-inoculation with native AMF? Is the AMF community of the roots altered by the pre-inoculation treatment? Are the effects of pre-inoculation different during spring and fall transplanting? Are increases in colonization associated with increases in seedling survival? It was anticipated that assessing these questions would not only increase our knowledge of the ecology and diversity of AMF in sagebrush habitats but also may lead to improved techniques for the establishment of Wyoming big sagebrush.

MATERIALS AND METHODS

Plant and Fungal Material

Seeds of *Artemisia tridentata* ssp. *wyomingensis* were obtained from the USDA/Rocky Mountain Research Station and the Bureau of Land Management in Boise, ID. The seeds provided by the USDA/Rocky Mountain Research Station were collected near Casper, Wyoming (42°30' N, 106°30' W) in 2009 and were used for the experiments with potted plants. The seeds provided by the Bureau of Land Management had been collected in 2010 near Big Foot Butte in southwestern Idaho (43°18'48.43"N, 116°21'48.57"W) and were used for the field experiments. Seeds were cleaned using an air-column blower to separate seeds from chaff and stored at 4°C until planting.

To produce mycorrhizal inoculum, sandy-loam soil was collected from Kuna Butte, Idaho (43°26.161'N, 116°25.848'W, 908 m a.s.l.), a sagebrush-steppe community on the western Snake River Plain south of Boise, Idaho. This community is dominated by *Artemisia tridentata* ssp. *wyomingensis*, *Poa secunda* (J. Presl.), *Elymus elymoides* (Raf. Sweezy), and the open interspaces are occupied by biological soil crusts. A previous study had shown that sagebrush plants at the Kuna butte site were colonized by AMF and that at least six AMF phylotypes were present at the site, with the most common ones being within the *Rhizophagus* and *Glomus* genera (Carter et al. 2014). Pot cultures were prepared as described by Morton et al. (2004) using Sudan grass (*Sorghum bicolor* L. var. *sudanense*) as a host. Briefly, I mixed soil samples collected at the field sites with

autoclaved sand (1:1) and transferred the mixture to 1.5 L pots. Sudan grass seeds were surface sterilized by soaking them in 70% ethanol for 1 min and 0.5% sodium hypochlorite for 20 min. After rinsing with deionized water, 100 seeds were planted per pot and the seedlings were grown in a greenhouse (15-h photoperiod and day/night conditions of $25/18 \pm 3$ °C) for three months. Pots were watered daily to field capacity and were fertilized weekly with a 1/8 strength Hoagland solution without phosphorus (P) and every four weeks with the same solution with P. After three months, watering was ceased and the pot cultures were allowed to dry and then the shoots were removed. The soil and roots remaining in the pots were used again to repeat the cycle from seeding to shoot removal two more times. After three cycles of pot culture cultivation, the soil and roots were stored in plastic bags and within four months used to inoculate sagebrush seedlings.

Mesocosm Experiments

Seedlings for these experiments were first grown in a greenhouse in 50 ml cone-tainers containing roots and soil from the pot cultures (pre-inoculated seedlings), or sterilized pot cultures (non-inoculated seedlings). After 3 months, the seedlings were transplanted to 24 L tree-pots filled with untreated soil from Kuna butte. This is a sandy loam soil with an available phosphorus content of $8.1 \mu\text{g g}^{-1}$ of soil. Two experiments were conducted. For the first experiment, the seedlings were transplanted during the spring of 2011 and for the second in early fall of the same year. For the spring transplanting experiment, 150 pots were used, half with non-inoculated seedlings and half with inoculated ones. After transplanting, plants were grown outdoors at the Idaho

Botanical Gardens (N 43° 35' 52.1" W 116° 9' 42.3") under ambient temperature and precipitation conditions. In addition, 10 other pots, 5 with non-inoculated and 5 with inoculated seedlings were grown under the same conditions but were irrigated to maintain the soil at field capacity. Pots were distributed at random and a weather station at the experimental site recorded temperature, relative humidity, and precipitation. Soil volumetric content was monitored with moisture probes (Echo 20; Decagon Devices, Inc., Pullman, WA) in 5 pots per treatment. I also measured seedlings' predawn water potential, which is indicative of the water potential of the soil occupied by the roots. Predawn water potential measurements were made using a pressure chamber (PMS Instrument Company; Albany, OR, USA). Seedling survival, shoot growth, and photosynthetic characteristics were measured biweekly. Mycorrhizal colonization was assessed just prior to transplanting and 2.5 and 5 months afterwards. Samples used for the analysis of AMF colonization were also used to extract DNA from roots and identify AMF phylotypes by molecular methods. At the end of the experiments some plants were used to estimate dry biomass and their leaves to determine the $^{13}\text{C}/^{12}\text{C}$ isotope ratio ($\delta^{13}\text{C}$).

The experiment started in early fall followed a similar approach, however 50 pots were prepared per treatment and none of the pots received additional water because of the absence of drought stress. Mycorrhizal colonization and composition were determined prior to transplanting and 7 months afterwards, survival at monthly intervals, and shoot growth 1, 7, and 8 months after transplanting. No measurements of photosynthetic characteristics were taken during this experiment.

In addition to the experiments described above, another experiment was initiated in 2012, with seedlings transplanted during the spring of this year. The approaches were similar to those described above, except that the pots were filled with untreated soil collected at the Big Foot Butte site. This is a silty-loam soil with a P content of $43 \mu\text{g g}^{-1}$ of soil. Fifty pots were used per treatment and the pots only received water from natural precipitation. The sole purpose of this experiment was to determine whether changes in the AMF community of inoculated seedlings occurred following transplanting. To achieve this goal, I analyzed the AMF community of inoculated seedlings prior to transplanting and three months afterwards.

Field experiments

Field studies were conducted in the spring and fall of 2012 near Big Foot Butte, ID ($43^{\circ}18'48.43''\text{N}$, $116^{\circ}21'48.57''\text{W}$), within the Morely Nelson Snake River Birds of Prey, National Conservation Area. The experimental site had experienced a fire during the previous summer that killed all the sagebrush plants. For the field experiments, I initially grew sagebrush seedlings for three months under the greenhouse conditions described above. However, rather than using cone-tainers to grow the seedlings I used Jiffy-7 peat pellets. After expansion, the center of the pellet was filled with either 6 ml of sterile soil from the field site (non-inoculated treatment), untreated soil from the field site (natural treatment), or soil from the pot cultures of native mycorrhizae (inoculated treatment). Before transplanting to the soil at the field site, seedlings were acclimated to outside conditions for two weeks. For this purpose, seedlings were moved outdoors during the day and brought back to the greenhouse at night. One hundred seedlings were

transplanted per treatment, 300 on April 12, 2012 (spring transplanting) and 300 on October 4, 2012 (fall transplanting). Seedlings were randomly planted on the North-East side of residual sagebrush stumps that remained after the fire; this placement is believed to shade seedlings during the afternoon and reduce water loss and heat stress. Seedlings in the jiffy pellets were placed in a small hole, the pellets were completely covered with soil, and the soil was well watered. Additional water was applied during the first two weeks after transplanting. During this period, the seedlings received 300 mL of water every 3 days to improve initial establishment. Following transplanting, I assessed AMF colonization, AMF community composition, and seedling survival. For measurements of AMF colonization and community composition, seedlings were collected 4 and 7 months after the spring and fall transplanting, respectively. Collection of seedlings involved digging a trench around the seedling with an approximate diameter of 1.5 meters and carefully excavating the soil to retain the intact root system. During these steps, particular care was taken to minimize damage to the fine roots, where most of the mycorrhizal colonization occurs. Samples were stored at -24 °C until processing. Due to the laborious nature of this work, I only collected seedlings from the non-inoculated and inoculated treatments.

Growth measurements

To estimate seedling growth, I measured shoot height, shoot width, and total plant biomass. Shoot growth was quantified by measuring the height of the tallest shoot as well as the greatest width of each seedling. These measurements were then used to calculate the shoot volume of each seedling using the equation for the volume of an

oblate ellipsoid ($4/3 \pi ab^2$, where a and b are half the height and width, respectively). For the determination of the shoot: root ratio and total biomass, shoots and roots were dried at 80°C until no changes in weight were detected between successive days. For the spring mesocosm experiment, the phenology of ephemeral leaf abscission was assessed by recording the percent of plants with ephemeral leaves on June 30, July 12, and July 26, 2011.

Chlorophyll a fluorescence measurements

Prior to the measurements of chlorophyll a fluorescence, leaves of *A. tridentata* growing at the Idaho Botanical Gardens were dark adapted overnight using dark adaptation clips. Chlorophyll a fluorescence was determined with a pulse amplitude modulated fluorometer (OS5p, Optosciences, Inc, NH). After dark adaptation, minimal fluorescence (F_0) was measured with a red light of $0.1 \mu\text{mol m}^{-2}\text{s}^{-1}$ and maximal fluorescence (F_m) following a pulse of saturating light of $8000 \mu\text{mol m}^{-2}\text{s}^{-1}$ and 0.8 second duration. The samples were subsequently exposed to actinic light of $600 \mu\text{mol m}^{-2}\text{s}^{-1}$ for 20 minutes provided by the halogen lamp of the OS5p. During this period, and at 2 minute intervals, the samples were exposed to saturating light flashes to estimate the maximal fluorescence (F'_m) and steady-state fluorescence (F'_s) of light-adapted leaves. Chlorophyll a fluorescence is a nondestructive technique that has been increasingly used to monitor plant responses to abiotic stress such as cold temperatures, water and nutrient deficits (Baker 2008; Cavender-Bares 2004), as well as the effects of biotic factors including AMF colonization (Pinior et al. 2005). From F_0 , F_m , F'_m and F'_s , four photosynthetic parameters were estimated: the maximum quantum efficiency of

photosystem II (PSII) photochemistry, $F_v/F_m = (F_m - F_o)/F_m$; the PSII operating efficiency, $\Delta F/F_m' = (F_m' - F')/F_m'$; the non-photochemical quenching, $NPQ = [(F_m/F_m') - 1]$; and the ratio of F_s' to F_o (Baker 2008). The F_v/F_m ratio represents the maximum proportion of light absorbed by photosystem II that can be used to drive photosynthesis (photochemistry) rather than being dissipated as heat or fluorescence. Values of F_v/F_m are measured in dark adapted leaves and various stresses tend to reduce these values (Cavender-Bares 2004). For an illuminated leaf, $\Delta F/F_m'$ measures the proportion of light absorbed by photosystem II that is used for photochemistry. The value of $\Delta F/F_m'$ is smaller than F_v/F_m because under illumination there is an increase in heat dissipation (NPQ), which helps to prevent photodamage caused by excess light. The $\Delta F/F_m'$ and NPQ values are affected by characteristics of the growing environment including light intensity and temperature (Cavender-Bares 2004; Flexas et al. 2000). The physiological significance of the F_s'/F_o is not clear, but this ratio tends to decrease in plants experiencing water deficits (Flexas et al. 2000). Measurements of fluorescence were made bi-weekly from June to October of 2011. At each time, I measured five plants per treatment.

Gas exchange measurements

Gas exchange measurements of *A. tridentata* leaves were made using a LI-6400-40 chamber connected to a LI-COR LI-6400XT portable photosynthesis system (LI-COR Inc, Lincoln, NE). For each sample, net photosynthetic carbon assimilation (net photosynthesis), stomatal conductance, transpiration, and leaf temperature were assessed. Net photosynthesis, stomatal conductance, and transpiration were measured at an

incoming air CO₂ concentration of 400 $\mu\text{mol mol}^{-1}$, 50 (± 5) % relative humidity, ambient temperature, and a saturating light intensity of 1700 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The infrared gas analyzer was matched prior to each measurement and values of net photosynthesis and transpiration were recorded after the CO₂ assimilation rates and stomatal conductance values became stable. Leaves were arranged to fully cover the leaf chamber area and this area was used to calculate the CO₂ assimilation and transpiration rates per unit area of each sample. From the values of CO₂ assimilation and transpiration, the LI-COR system estimated stomatal conductance which was then used to calculate photosynthetic water use efficiency. Measurements of gas exchange were taken, on five plants per treatment, bi-weekly from June to October of 2011.

Carbon isotope discrimination

To ascertain whether AMF colonization affected water use efficiency, I also measured foliar carbon isotope ratios ($\delta^{13}\text{C}$). In all experiments, leaf samples were collected when plants were harvested for analysis of AMF colonization. For each sample, 1.75 mg of leaf material was oven dried and ground in 4 mL vials having 2 copper plated balls (H&N sport 4.50 mm BB). Vials were shaken twice for 5 minutes each on a Tornado II portable paint shaker. Subsequently, samples were processed through an elemental combustion system (ECS 4010 CHNS-O Elemental Analyzer, Costech Instruments) and the combustion products were separated through a reduction column followed by a water trap and gas chromatography column before being analyzed using a Cavity Ring Down Spectrometer (CRDS) isotope analyzer (Picarro G2101-i gas

analyzer). Spinach, glycine, powdered sugar, and caffeine were used as standards to ascertain consistent calibration of the equipment throughout these measurements.

AMF Colonization

The effect of inoculation on AMF colonization of *A. tridentata* roots was assessed just prior to transplanting and 3, 4, or 7 month afterwards depending on the experiment. A minimum of five plants were analyzed for each sampling time and treatment. Roots were cut into 0.5 to 2 cm segments, placed in 5% Potassium hydroxide (KOH), and cleared in an autoclave by heating at 121°C for 5 minutes. Subsequently, the roots were rinsed with water, immersed in 1% Hydrochloric acid (HCl) for 20 min, and then stained overnight with 0.03% Chlorazol black E (CBE) in lactoglycerol (1:1:1 water:glycerin:lactic acid). Roots were de-stained for 1 to 2 minutes with dimethyl sulfoxide (DMSO) to reduce non-specific staining, rinsed with water, and stored in 50% glycerol. Fine root segments were mounted on slides and viewed at 200X magnification. When needed, the presence of AMF structures was ascertained by observing the samples at 400X magnification. Total mycorrhizal colonization and the percent root length containing arbuscules and vesicles were estimated by the intersection method using 200 intersections per sample (McGonigle et al. 1990).

DNA isolation, amplification, cloning, and sequencing

For plants that were analyzed for AMF colonization, I also extracted DNA from the roots to determine the dominant phylotypes present in each plant. Fine lateral roots (<2 mm) were thoroughly rinsed in nanopure water to remove soil particles.

Subsequently, the roots were ground and the DNA extracted from them using a cell disrupter (Thermo Savant FastPrep® FP120) and the FastDNA® Green Spin Kit as described by the manufacturer (MP Biochemicals). DNA was amplified via nested Polymerase chain reaction (PCR) using the general fungal primers LR1 [5'-GCA TAT CAA TAA GCG GAG GA-3'] and FLR2 [5'-GTC GTT TAA AGC CAT TAC GTC-3'] in the first amplification and the Glomeromycota specific primers, FLR3 (5'-TTG AAA GGG AAA CGA TTG AAG T-3') and FLR4 (5'-TAC GTC AAC ATC CTT AAC GAA-3') in the second (Gollotte et al. 2004; Van Tuinen et al. 1998). This procedure amplifies the D2 region of the 28S ribosomal RNA gene (LSU-D2). For both amplifications, PCR reactions were performed using 12.5 µl of GoTaq® hot start colorless master mix (Promega), 6.25 pmol of each primer, 2.5 µl of DNA template or 2.5 µl of 1:10 dilution of the first PCR product, and nuclease free water to a final volume of 25 µl. Thermocycler conditions were as follows: 5 minutes initial denaturation at 94°C; 28 cycles of 1 minute denaturation at 94°C, 1 minute annealing at 58°C, and 1 minute extension at 72°C; followed by a final extension at 72°C for 10 min. After the second amplification, PCR products were cloned with the pGEM®-T easy vector system (Promega) following the manufacturer's instructions, except that only half of the specified volumes were used. Ligation reactions were incubated overnight at 4°C to maximize ligation efficiency. The vectors were then heat transformed into high efficiency competent cells of *Escherichia coli* (strain JM109). The cells were spread onto Luria-Bertani agar plates containing 100 µg ml⁻¹ ampicillin and supplemented with IPTG and X-Gal. Positive transformants (white colonies) were selected and the inserted region amplified via PCR with the vector specific primers SP6 and T7. Approximately 15

positive transformants were selected from each sample. The PCR products were cleaned using ExoSAP-IT (Affimetrix), diluted with nanopure water, and sent for sequencing to a commercial facility (Genewiz, Inc.).

Phylogenetic Analyses

Forward and reverse sequences were first assembled into a consensus sequence and then proofread and trimmed to only include the area amplified by the FLR3/FLR4 primer set using Geneious Pro version 5.4 (Biomatters Ltd.). Sequences were compared to available GenBank sequences using the BLAST tool to determine that they were from Glomeromycota, and to identify closely matching sequences based on the origin and best scoring hit in GenBank. Initial alignment was performed with ClustalW2 and manual adjustment and review was performed using Phyde® v0.9971. In total 710 unique sequences were used in the full alignment. Additionally, 244 sequences were used from Carter et al. (2014) as well as 30 sequences from GenBank and from Krüger et al. (2012). The latter were downloaded from the AMF phylogeny website

(<http://schuessler.userweb.mwn.de/amphylo/>).

Maximum parsimony analyses were performed using PRAP2 (Muller 2004) in conjunction with PAUP*4.0b10 (Swofford 2002). Bootstrap support (BS) for nodes was estimated with 1,000 heuristic replicates using PRAP2 (Felsenstein 1985). Descriptive statistics reflecting the amount of phylogenetic signal in the parsimony analysis were given by the consistency index (CI), retention index (RI), and the resulting rescaled

consistency index (RC) (Farris et al. 1994). Bayesian analyses were run with MrBayes 3.2. Molecular evolutionary models for Bayesian analysis were estimated with jModelTest (Guindon and Gascuel 2003; Posada 2008). The best fit model was GTR+I+G. Bayesian analyses were performed using four to one heated chains for ten million generations. Convergence was determined by viewing in Tracer v1.3 (Rambaut and Drummond 2005) and burn in of 50,000 generations was discarded prior to sampling the posterior distribution.

After phylogenetic analyses, sequences were assigned to different phylotypes if they consistently separated into distinct monophyletic groups and left no sequence outside a strongly supported clade. This is a conservative approach that may underestimate the number of phylotypes, but avoids the presence of paraphyletic groups in the cladograms. The phylotypes were designated after the major mycorrhizal clades in which they were placed, followed by a numerical index (Appoloni et al. 2008). To characterize genetic differences that may not be resolved in the phylogenetic analysis, sequences were also grouped into operational taxonomic units (OTUs) with sequence similarities $\geq$ than 94% using the Mothur program (Schloss et al. 2009; Wang et al. 2011). These OTUs were assigned to phylotypes and identified based on the phylotype name and an additional numerical index.

Data analyses

For two level analyses such as the comparison of AMF colonization, shoot volume, biomass, and $\delta^{13}\text{C}$ between non-inoculated and inoculated seedlings, the data were analyzed using an independent *t*-test or a Welch *t*-test if variances were equal and

unequal, respectively. The effect of inoculation, week of measurement, and the interaction between these factors on gas exchange and chlorophyll fluorescence parameters were analyzed by two-way ANOVA. Prior to statistical analysis, the normality and homoscedasticity of the data were examined by the Shapiro-Wilk's and Levene's test, respectively. Values of F_v/F_m , $\Delta F/F_m'$, NPQ , and stomatal conductance were not normally distributed; these values were Box-Cox transformed to generate a normal distribution. Significant differences among treatments were determined using Tukey-Kramer least square means test at $P < 0.05$. Differences in survival among treatments were evaluated using a logrank test (Sigmaplot 12.5).

The AMF community of individual seedlings was estimated based on the frequency of clones of each OTU detected. Biases in PCR amplification can lead to relative clone abundances that are different from the relative abundance of sequences in the original samples (Liesack et al. 1991; O'Mullan and Ward 2005). However, PCR biases are likely to be similar in different samples, a sequence that is preferentially amplified from DNA in one sample is likely to experience the same bias during amplification from another sample. Thus, clone counts can provide a reasonable estimate of the relative abundance of OTUs in different samples (Liu et al. 2011; O'Mullan and Ward 2005; Wang et al. 2011). Based on clone counts of OTUs, the OTU richness is reported (S =average number of unique OTUs per plant) as well as calculation of the Shannon index of diversity ($H = -\sum_{i=1}^S p_i \ln p_i$, where p_i is the proportion of sequences belonging to each phylotype relative to the total number of sequences).

In addition, possible differences in OTU composition were analyzed between non-inoculated and inoculated seedlings using non-metric multidimensional scaling

(NMDS). NMDS is an unconstrained ordination method that arranges communities according to their similarities in species composition (Minchin 1987). The NMDS ordination was conducted using the metaMDS function in the vegan package in R (Oksanen et al. 2007). The similarity matrix for the NMDS was calculated using the Bray-Curtis coefficient on OTU counts. To test the effect of inoculation on the AMF community, I used the envfit function in vegan with non-inoculated/inoculated seedlings as categorical variables. The envfit function was also used to compare the AMF communities before and after transplanting as well as those of samples collected in different seasons. The envfit function provides a goodness of fit and p -value based on permutation tests, which allowed assessing the significance of the relationship of each variable to the AMF community. Except for the survival analysis, all statistical tests were conducted in R (R Core Team 2012).

RESULTS

Mesocosm Experiments

Transplanting in spring 2011

Following transplanting, the seedlings were exposed to natural climatic conditions from May 6 to October 6, 2011 (Fig. 1). During the first 45 days after transplanting average daily temperatures gradually increased from about 10 to 15 °C and cumulative precipitation was 76 mm, which was sufficient to maintain soil moisture above 15%. From late June to the end of the experiment, average daily temperatures tended to be above 20 °C with maximum temperatures reaching 38 °C. During this period precipitation was rare with a combined precipitation in July, August, and September of about 5 mm. Ten pots were watered daily to maintain the soil at field capacity, which corresponded with a volumetric soil moisture content of about 20%. The other 150 pots only received natural precipitation, except during the last week in July; when they were supplemented with a total of 2.5 mm of water. The volumetric water content of the soil in these pots ranged from 0 to 10% and no difference in soil water content was detected between non-inoculated and inoculated seedlings (data not shown). When compared with average values for Boise (National Oceanic and Atmospheric Administration)(NOAA/NCDC 2014), precipitation during the first two months of the experiment was somewhat higher than normal, 76 and 53 mm, respectively. In contrast for the last three months of the

experiment, precipitation was lower than normal, receiving 8.89 mm compared to the 120 year average of 31.5 mm during this period.

At the time of transplanting, AMF colonization of non-inoculated seedlings was negligible. In contrast, inoculated seedlings showed a total colonization of 57.6 ($\pm$ 7.08) % and an arbuscular colonization of 6.7 ($\pm$ 2.79) % (Fig. 2A). Differences in colonization were also observed 2.5 months after transplanting. Total colonization of non-inoculated seedlings was lower than that of inoculated ones, 19.8 ($\pm$ 3.36) and 43.99 ($\pm$ 6.28) %, respectively. Similarly, arbuscular colonization was lower in non-inoculated than inoculated seedlings, 2.99 ($\pm$ 0.78) and 10.2 ($\pm$ 3.95) %, respectively. On October 6, 2011, when the Spring 2011 experiment was ended, difference in total colonization were maintained; 18.74 ($\pm$ 1.69) and 49.59 ($\pm$ 7.40) % for non-inoculated and inoculated seedlings, respectively. However, no statistical differences were noted for arbuscular colonization despite relative large differences in the mean values, 1.36 ($\pm$ 0.52) and 16.7 ($\pm$ 8.13) % for non-inoculated and inoculated seedlings, respectively. Colonization in the well-watered controls was also assessed. No differences were observed in total colonization between non-inoculated and inoculated seedlings, 20.8 ($\pm$ 5.1) and 25.3 ($\pm$ 8.0) %, respectively.

Independent of the treatment, no mortality occurred until the middle of July, but subsequently mortality was higher in non-inoculated than inoculated seedlings. On October 6, survival was 52 and 76 % for non-inoculated and inoculated seedlings, respectively, and 100% in the well-watered plants (Fig. 2B). By the beginning of September, differences in survival among treatments became apparent. To determine if these differences were related to differences in soil and plant water status, I measured

predawn water potentials on September 9. At this time, the values of predawn water potential were $-0.63 \text{ MPa } (\pm 0.06)$ for the well-watered seedlings, $-2.76 \text{ MPa } (\pm 0.49)$ for the non-inoculated seedlings, and $-3.08 \text{ MPa } (\pm 0.55)$ for the inoculated ones. Thus, the well watered seedlings had higher predawn water potential than those under natural precipitation ($p < 0.05$), while no differences occurred between the non-inoculated and inoculated treatment.

Notwithstanding the effect on survival, inoculation of sagebrush seedlings did not have a significant effect on seedling growth as measured by seedling volume or dry biomass (Fig. 2C, Table 1). For seedlings collected in July or October, the dry biomass and shoot-to-root ratio of non-inoculated seedlings did not differ from those of inoculated ones. The shoot to root ratio was neither affected by watering (Table 1); however, well watered plants grew much more than those receiving natural precipitation. At the end of the experiment, the shoot volume and total biomass were 3 to 4 fold higher in well watered plants than those receiving natural precipitation (Fig. 2C, Table 1). In addition, the timing of ephemeral leaf senescence was postponed in the well-watered plants (Fig. 3); based on the log rank test, this effect was statistically significant ($p < 0.001$). In contrast, no differences in the phenology of ephemeral leaf senescence was detected between non-inoculated and inoculated seedlings ($p = 0.794$).

To assess the effect of inoculation on plant physiological characteristics, various chlorophyll fluorescence and gas exchange parameters were measured. The maximum quantum efficiency (F_v/F_m) and operating efficiency ($\Delta F/F_m'$) of photosystem II were not affected by the treatments (Table 2). For F_v/F_m and $\Delta F/F_m'$, there was a significant interaction between the date when the measurements were made and the treatment

applied. However, when comparisons were made within a specific date, no differences were detected among any of the treatments. In contrast, significant differences were observed for *NPQ* and the *F_s/F_o* ratio (Table 2). The non-inoculated seedlings showed higher *NPQ* values than the well-watered plants, while no differences were apparent between the inoculated seedlings and those in the other treatments (Fig. 4A). Differences were also observed for *F_s/F_o*; the non-inoculated seedlings had lower values than the well-watered plants, while no differences were noticed between the inoculated and non-inoculated seedlings or between inoculated seedlings and well-watered ones (Fig. 4B).

Net photosynthesis, stomatal conductance, and water-use efficiency (WUE) varied through the experimental period and among treatments, though no significant interaction occurred between these two factors (Table 2). Among treatments the differences were mainly attributed to watering. The well watered plants had higher rates of photosynthesis than the non-inoculated seedlings (Fig. 5A). In contrast, no significant differences were detected between inoculated seedlings and those of the other two treatments. The well-watered plants had also higher stomatal conductance and lower water-use efficiency than those receiving natural precipitation (Fig. 5B and C). Differences in stomatal conductance and water-use efficiency between non-inoculated and inoculated seedling were not significant.

The values of foliar carbon isotope ratios ($\delta^{13}\text{C}$) were consistent with the results of WUE obtained using the portable gas exchange system. The well-watered plants had significantly lower $\delta^{13}\text{C}$ values (less intrinsic water use efficiency) than those receiving natural precipitation while no differences were detected between non-inoculated and inoculated seedlings (Fig. 6).

Transplanting in Fall 2011

During the first 60 days after transplanting, average daily temperatures gradually decreased from about 15 to 0°C and cumulative precipitation was approximately 50 mm. In December and January, temperatures ranged from -5 to 10°C and the highest rate of precipitation in the form of rain and snow occurred in the second part of January totaling 81 mm. From the middle of February to the beginning of June, temperatures gradually increased from about 3 to 20°C and cumulative precipitation during this period was 122 mm. When compared with average values for Boise (NOAA/NCDC 2014), precipitation during the first four months of the experiment were normal at 143.51 mm, Boise typically receiving an average of 155.96 mm. Similarly, average precipitation was observed from February to June, 2012, totaling 157 mm compared to the 120 year average of 162.31 mm for this period.

The effect of inoculation on AMF colonization was similar to that observed in the spring transplanting experiment. At the time of transplanting on October 1, total and arbuscular colonization of non-inoculated seedling was negligible, 0.17 and 0 %, respectively. In contrast, inoculated seedlings had a total colonization of 69.06 % (± 0.64) and an arbuscular colonization of 18.07 % (± 11.3) (Fig. 8A). In May 2012, seven and one-half months after transplanting, inoculated seedlings maintained higher levels of AMF colonization than non-inoculated ones. The latter had a total colonization of 23.12 % (± 5.57) and an arbuscular colonization of 5.74 % (± 2.35), while total and arbuscular colonization of inoculated seedlings was 43.80 % (± 3.36) and 12.08 % (± 2.57), respectively.

Even though inoculation increased AMF colonization, it did not affect the other parameters measured. Seedlings transplanted in the fall were monitored for survival for a period of nine months, (Fig. 8B). No seedling mortality was observed for either treatment until June, 2012. Survival was not found to differ as a result of inoculation and the experiment was terminated at the end of June when the size of the pot limited root growth. Estimates of shoot volume taken at the beginning, at the period of maximum ephemeral leaf expansion, and following ephemeral leaf senescence at the end of the experiment showed that inoculation did not affect shoot growth (Fig. 8C). Similarly, no differences were detected between non-inoculated and inoculated seedling on total dry weight, the shoot to root ratio, and $\delta^{13}\text{C}$ (Table 3).

Comparison between spring and fall transplanting

The environmental conditions that the seedlings experienced following spring and fall transplanting were markedly different. This appeared to have led to differences in growth patterns and intrinsic water use efficiency. Under natural precipitation, seedlings transplanted in spring had a rate of shoot growth of about 380 cm³ per month, while those transplanted in fall had a rate of about 750 cm³ per month. Furthermore, the shoot to root ratio was lower for seedlings transplanted in the spring than those transplanted in the fall, 1.75 (± 0.18) and 3.42 (± 0.37), respectively. As judged by the $\delta^{13}\text{C}$ values, seedlings transplanted in spring had also higher intrinsic water use efficiency than those transplanting in fall. Seedlings transplanted in spring that only received natural precipitation had $\delta^{13}\text{C}$ of -26.76 (± 0.34), while those transplanted in fall had $\delta^{13}\text{C}$ of -

28.77 (± 0.29). Differences in both the shoot to root ratio and $\delta^{13}\text{C}$ were statistically significant ($p < 0.001$).

Field Experiments

Three month old seedlings were transplanted into the soil near Big Foot Butte within the Morley Nelson Snake River Birds of Prey, NCA on April or October 2012. The spring of 2012 began with near normal snowpack and precipitation. Similarly, the period from April to the beginning of June also experienced near normal precipitation (80.5 mm) and average temperatures (14.2 °C). In contrast, total precipitation from the beginning of June to the end of September, was less than 1.5 mm. From June to September average temperatures ranged between 18 and 30°C. Overall, the period from June through September was one of the 10 driest and warmest on record (NOAA/NCDC 2014). In September temperatures began to decrease and reached a minimum in January 2013, when the average temperature was -9.1 °C. January was one of the 10 coldest observed over the 120 year period reported by the National Climate Data Center. The period from September 2012 through February 2013 received near normal precipitation, which totaled 145.4 mm. No additional precipitation was measured onsite between March 1 and October 4, 2013. Average temperatures rose from 5.3 °C in March to an average of 20.3 °C for June. July and August average temperatures were 27 and 24.8 °C, respectively, which set a new record for the hottest average temperatures for this period. Interestingly, the period from May to September set the record for the warmest 5 month period to date for Boise, Idaho.

Spring transplanting

The field experiments utilized jiffy peat pellets for the initial growth of the seedlings and included non-inoculated, natural, and inoculated treatments. At the time of transplanting, total and arbuscular colonization of non-inoculated seedlings was negligible, the natural treatment had intermediate values, and the inoculated treatment had the highest percent total and arbuscular colonization (Table 4). Notwithstanding these differences, the overall effect of inoculation on AMF colonization was less pronounced than in previous experiments. Three and a half months after transplanting, colonization was only assessed in non-inoculated and inoculated seedlings. At this time, the seedlings had developed a deep tap root, often up to 1 m in depth. I hypothesized that the position of the root within the soil profile may affect colonization due to differences in soil nutrients and moisture. To test this notion, colonization was evaluated separately in roots collected within the top 20 cm of the soil (shallow roots) and in roots collected below 30 cm (deep roots). Independent of the root location, no difference in total or arbuscular colonization were detected between non-inoculated and inoculated seedlings (Fig. 10 A and B). However, differences in colonization were evident between shallow and deep roots. Total colonization in the shallow roots were lower than in the deep roots ($p < 0.001$). After combining results from non-inoculated and inoculated seedlings, the total colonization of shallow and deep roots were $12.7 (\pm 2.8)$ and $41.4 (\pm 4.8)$ %, respectively. Differences were also noted for arbuscular colonization, although these values were very low $0.5 (\pm 0.2)$ and $2.1 (\pm 1.1)$ % for shallow and deep roots, respectively ($p = 0.02$).

For samples collected on July 31-2012, analysis of foliar $\delta^{13}\text{C}$ revealed no differences between treatments. Non-inoculated and inoculated seedlings had values of $-27.51 (\pm 0.27)$ and $-27.06 (\pm 0.50)$ ‰, respectively.

Following transplanting, the rate of mortality was rather uniform through the late spring and summer and no significant differences in survival were observed among treatments (Fig. 10C). By the beginning of October, the percent survival was 40, 50, and 50% for the non-inoculated, natural, and inoculated treatment, respectively. No mortality was observed during fall 2012 and winter and early spring of 2013. However, considerable mortality occurred during late spring and first part of the 2013 summer. As a result of this mortality, survival by October 2013 was about 20 % in all treatments.

Fall transplanting

At the time of transplanting total and arbuscular colonization of non-inoculated seedlings was negligible, the natural treatment had intermediate values, and the inoculated treatment had the highest percent total and arbuscular colonization (Table 4). These differences in colonization were similar to those observed prior to spring transplanting. One difference was, however, the level of colonization in inoculated seedlings. At the time of fall transplanting, the inoculated seedlings had somewhat higher levels of colonization than those observed in spring (Table 4). About 8 months after transplanting, roots were collected from non-inoculated and inoculated seedlings. These roots were mainly in the upper 20 cm of the soil and no attempts were made to distinguish between shallow and deep roots. In contrast to the spring experiment, analysis of colonization revealed differences between inoculation treatments. Total colonization of

inoculated seedlings was twice that of non-inoculated seedlings (Fig. 10A). Differences were also observed in arbuscular colonization. Average arbuscular colonization for non-inoculated and inoculated seedlings was 0.5 and 5%, respectively; these values were significantly different ($p < 0.001$) based on Wilcoxon rank sum test.

Samples for analysis of foliar $\delta^{13}\text{C}$ were collected on May 20, 2013. No differences were observed between treatments. Non-inoculated and inoculated seedlings had values of $-28.40 (\pm 0.22)$ and $-28.22 (\pm 0.32) \text{‰}$, respectively. These values of foliar $\delta^{13}\text{C}$ were significantly lower than those measured in leaves collected in midsummer from spring-transplanted seedlings ($p = 0.012$). After combining results from the non-inoculated and inoculated treatments, seedlings transplanted in spring had $\delta^{13}\text{C}$ of $-27.29 (\pm 0.28)$, while those transplanted in fall had $\delta^{13}\text{C}$ of $-28.40 (\pm 0.20)$.

Seedlings transplanted in October 2012 experienced mortality through the fall and winter (Fig. 11). At the beginning of spring survival was about 50, 67, and 85% for the non-inoculated, native, and non-inoculated treatments, respectively. Mortality continued to increase during the spring and summer. By the end of the spring, survival was 18, 26, and 41% for the non-inoculated, native, and non-inoculated treatments, respectively. Similarly at the beginning of fall, one year after transplanting, the non-inoculated seedlings had the lowest survival rate at 4%, the native treatment an intermediate value of 11%, and the inoculated seedlings the highest survival rate at 31%. Among all treatments, these differences in survival were significant.

Phylogenetic Analyses

The maximum parsimony and Bayesian analyses produced trees with similar structure and a representative tree using maximum parsimony topology with corresponding bootstrap support values is presented (Figure 12). The 710 unique sequences obtained were all within the Glomerales (Redecker et al. 2013); about 5% of the sequences belonged to the family Claroideoglomeraceae and the rest to the Glomeraceae. Within the Claroideoglomeraceae, the phylogenetic analysis revealed two phylotypes, *Claroideoglomus I* and *II*. Sequences within phylotype *Claroideoglomus I*, OTU 1, shared greater than 94% sequence similarity with *Claroideoglomus claroideum* (N. C. Schenck & G. S. Sm.)(C. Walker & A. Schüßler, AJ271927); while the remaining sequences had up to 99% similarity with several published sequences of uncultured glomeromycetes (Carter et al. 2014; Mummey and Rillig 2007; Verbruggen et al. 2010). Within the Glomeraceae, the analysis resolved four phylotypes. Two phylotypes were within the *Glomus* genus and were designated *Glomus I* and *Glomus II*. Sequences within *Glomus I* shared greater than 94% similarity with *Glomus macrocarpum* (Tul. & C. Tul.) (C. Walker & A. Schüßler, FR750526). *Glomus II* was the phylotype with more sequences. A few of these sequences clustered with *Glomus microaggregatum* Koske, Gemma & P.D. Olexia. The majority of the sequences within *Glomus II* however did not cluster with known species, but showed high similarity with published sequences of uncultured and unnamed glomeromycetes (Carter et al. 2014; Mummey and Rillig 2007; Torrecillas et al. 2012b). Sequences were also identified within the *Funneliformis* and *Rhizophagus* genera. The former clustered with sequences obtained from a single spore of *F. mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler (FR750024) or isolates

of *F. mossea* (GQ330787). Similarly, sequences within the *Rhizophagus* phylotype clustered with *Rhizophagus intraradices* (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüßler, or *R. irregularis* (Błaszk., Wubet, Renker & Buscot) C. Walker & A. Schüßler.

The phylotypes obtained by the Bayesian analysis were compared with results from pairwise distance analysis (Figure 12). There was a good agreement between the results of these analyses; a particular OTU was only present in one phylotype. On the other hand, all phylotypes included more than one OTU. The largest number of OTUs was detected in *Rhizophagus* followed by *Glomus II*; which had eight and seven OTUs, respectively. *Glomus II* included sequences showing pairwise differences of up to 22%. Considerable genetic variation was also observed in the other phylotypes. *Funneliformis*, *Glomus I*, and *Claroideoglomus I* and *II* contained 5, 4, 3, and 2 OTUs, respectively. Furthermore, pairwise differences within these phylotypes ranged from 12% in *Claroideoglomus II* to 25% in *Funneliformis*.

AMF community composition in non-inoculated and inoculated seedlings

To evaluate whether inoculation altered the AMF community of the roots, I compared the richness and diversity of OTUs in non-inoculated and inoculated seedlings. Within a particular experiment, no differences were detected in the richness index (S) between non-inoculated and inoculated seedlings (Table 5). Among experiments, the average S values ranged from 3 to 6, indicating that most seedlings were colonized by AMF in more than one OTU. However, the Shannon index of diversity (H') was much lower, suggesting the predominance of certain OTUs over others (Table 5). The H' values did not differ between inoculation treatments except for the spring 2012 field

experiment; in this experiment, H' was somewhat higher in non-inoculated than inoculated seedlings. H' values calculated for seedlings at the time of transplanting into the spring 2012 mesocosm experiment (“Pre-transplant” in Table 5) were significantly higher than both inoculated and non-inoculated seedlings 3 months after transplanting.

Although richness and diversity were similar, the particular OTUs colonizing the roots could have differed between treatments. This possibility was analyzed by nonmetric multidimensional scaling. For both the mesocosm and field experiments, NMDS plots showed no significant differences between non-inoculated and inoculated treatments (Fig. 13). In addition for the spring 2012 mesocosm experiment, I compared the AMF community composition of inoculated seedlings just before transplanting to that of seedlings collected three months afterwards. These communities showed little separation in the ordination space and their centroids were not significantly different ($p = 0.82$) (Fig. 14A). Similarly, the results from the spring field experiment indicate that the depth of the rooting within the soil did not have an influence on the AMF community; the location of the centroids and 95% confidence intervals of AMF communities of shallow and deep roots were virtually identical (Fig. 14B, $p = 0.89$).

For the 2011 mesocosm experiments and the 2012 field experiments, I also compared the AMF communities of seedlings harvested during the summer (4 to 5 months after spring transplanting) to those harvested in early spring (7 to 8 months after fall transplanting). Seedlings from the mesocosm experiments showed more seasonal variation in their centroids than those from the field experiments (Fig. 14 C and D). However, in both analyses the centroids were not significantly different with p values of 0.16 and 0.73 for the mesocosm and field experiments, respectively.

The lack of significant differences in AMF communities between inoculation treatments, root depth, and seasons appeared to have been related to the predominance of certain OTUs. In all experiments and treatments, the most common OTU was *GII-1* (Fig. 15). Overall this OTU was detected in 94% of the seedlings (Fig. 15 F). Other OTUs that were present in relatively high frequency were *F1*, *GII-2*, and *R1*, which were detected in 54, 51, and 40% of the seedlings respectively. For these OTUs similar patterns were, in general, observed in individual experiments. *Funneliformis 1* and *GII-2* were found in more than 50% of the seedlings in 4 of the 5 experiments, the exception was the fall 2011 experiment, where only 18% and 31% of the seedlings had *F1* and *GII-2*, respectively. The fourth most abundant OTU, *R1*, showed more variation. In the mesocosm experiments (Fig. 15 A, B, and E), *R1* was found in at least half of the seedlings. In contrast, in the field experiments, *R1* was not detected in one of the treatments or it was found at low frequency on the other (Fig. 15 C and D). Another major factor contributing to the dominance of certain OTUs over others is that several OTUs were only found in very few seedlings. Specifically, 15 of the 29 OTUs were detected in 3 or less of the 68 seedlings analyzed and ten of them in only 1 seedling. Thus, the overall contribution of these OTUs to the AMF community of sagebrush seedlings appears minimal.

DISCUSSION

Pre-inoculation effects on mycorrhizal colonization after transplanting

In natural habitats, sagebrush seedlings are colonized to varying degree by AMF present in the soil (Busby et al. 2011; Gurr 1993). However, damage to fungal networks by soil disturbances and limited root development at the seedling stage tend to reduce the chances of encountering AMF propagules, thereby decreasing the probability of colonization (Klironomos et al. 1993). Based on the results of my experiments, the amount of AMF propagules present in the two studied soils was a limiting factor to colonization. Several months after transplanting, colonization in non-inoculated seedlings was about half of that observed in pre-inoculated ones. This occurred in four of five experiments, the only exception being the field experiment started in the spring of 2012.

Mycorrhizal colonization is an ongoing process; the fungus retreats or is excluded from older portions of the root, while younger portions become colonized by AMF (Bonfante and Perotto 1995). Intra-radical hyphae may extend into the elongating portions of the root. However, most often, maintenance of colonization requires repeated infections, which occur predominately behind the root tip (Allen 2001). Host reinfection by AMF can arise from germination tubes emerging from spores, extra-radical runner hyphae connected to the intact root system, or hyphae arising from broken root fragments (Friesse and Allen 1991). In my experiments, the seedlings were transplanted with the sand/soil mixture in which they grew while in the greenhouse. For the pre-inoculated

seedlings, this mixture contained extra-radical hyphae, spores, and root fragments of Sudan grass. Consequently, all of these sources most likely contributed to the colonization of sagebrush roots that developed after transplanting. As the roots grew and the root tips became increasingly distant from the position of transplanting, higher colonization and density of extra-radical hyphae in the pre-inoculated seedlings may have resulted in higher propagule density near their root tips than near the root tips of non-inoculated seedlings; thus, maintaining differences in colonization even after several months of growth.

The persistent effect of inocula on colonization has been observed in other plants growing in semiarid habitats. One year after transplanting, seedlings of the tree *Cupressus atlantica* pre-inoculated with AMF native to an area in Morocco had 65% colonization (Ouahmane et al. 2007). In contrast, non-inoculated seedlings had only 25% colonization. Similarly, in an arid area in Spain, pre-inoculated seedlings of *Anthyllis cytisoides* had, 10 months after transplanting, 20% higher colonization than non-inoculated seedlings (Requena et al. 2001). This difference was observed with native AMF inoculum, but not with a commercial, non-native one. Even though the effect of non-native inoculum on promoting colonization tends to be less than that caused by native AMF (Weinbaum et al. 1996), some increases in colonization using non-native, commercial inoculum had been observed. For example, Gurr (1993) inoculated Wyoming big sagebrush seedlings with a commercial inoculum that was reported to only have one AMF species, *Glomus intraradices*. Non-inoculated and pre-inoculated seedlings were transplanted to a burned site 20 miles east of Boise, Idaho. Five months after transplanting, pre-inoculated seedlings had higher levels of colonization than non-

inoculated ones. However, the overall levels of colonization were low, 2.5 and 25% for non-inoculated and inoculated seedlings, respectively. Furthermore, no differences in colonization were observed one year after transplanting. Notwithstanding this lack of a long term effect, Gurr's results indicate that the use of commercial inoculum can cause an increase in colonization when the natural level of colonization is very low.

For Wyoming big sagebrush, few studies have analyzed natural levels of colonization. The lowest levels of colonization were reported by Gurr (1993) in areas heavily invaded by *Bromus tectorum*, with values of 5 and 2.5% prior to and after a fire, respectively. However, these values varied with the time of sampling; percent colonization was lower in seedlings collected in October than in those collected in June, when colonization was about 20%. Interestingly, higher levels of colonization were reported in a mine reclamation site, where attempts to recover the soil were started in 1983 (Stahl et al. 1988). Two and 14 years after the beginning of this process, colonization of Wyoming big sagebrush seedlings was about 18% and 80%, respectively (Frost et al. 2001). However, for big sagebrush in general, high levels of colonization seem to be rare. In two sites near Reno, Nevada, mature plants of *A. tridentata* ssp. *tridentata* and ssp. *vaseyana* had total colonization values ranging from 5 to 35% (Trent et al. 1994a). Overall, percent colonization was higher in ssp. *vaseyana* than *tridentata*, which may have been associated with higher moisture at the *vaseyana* site. A recent study also analyzed AMF colonization in seedlings of an unspecified subspecies of *Artemisia tridentata* (Busby et al. 2013). This study included five sites in Colorado, Utah, and Wyoming and the values of colonization reported for these sites ranged from 21 to 33%. In my mesocosm experiments, the levels of colonization without inoculation were

18.7 and 12.1% for seedlings collected in October and May, respectively; while in the field experiments, the natural level of colonization was 32 and 18% for seedlings collected in July and April, respectively. Thus the natural extent of colonization observed in my experiments appears to be within the range of values most commonly reported for sagebrush habitats.

For big sagebrush, AMF colonization has been reported to be lower during mid to late summer than at other times of the year (Gurr 1993; Trent et al. 1994a). However, I did not observe such a trend. In the mesocosm experiments colonization of seedlings were measured in mid-July, the beginning of October, and in May. For both non-inoculated and inoculated seedlings, total and arbuscular colonization were not significantly affected by the time of sampling. Similarly, for the field experiment, I analyzed samples collected at the end of July and in mid-April. The time of sampling did not have an effect on AMF colonization. The reasons for the discrepancies with other studies are not clear, but they may be attributed to differences in soil moisture or in the depth at which the samples were collected. In the mesocosm experiments, the roots were collected from the entire pot, which extended to a depth of 80 cm. Furthermore, in the field, roots during the summer were collected to a depth of up to 1 m. In contrast, Trent et al. (1994a) collected samples from only the upper 20 cm of the soil. Sampling a higher fraction of the soil profile may have prevented us from detecting differences that occurred in the upper soil layer, which is the layer that experiences greater fluctuations in moisture and temperature. Some support for this pattern comes from results of the field experiment. When only the colonization of roots collected in the upper 20 cm of the soil is considered, roots of inoculated seedlings collected during the summer had lower

colonization than those collected during the spring, 13.1 and 32.3%, respectively ($p = 0.012$). However, this was not observed for the non-inoculated seedlings. The overall lack of seasonal effects on colonization, particularly for the field experiment, may also reflect that the two sampling dates were not the extremes of possible annual variation in colonization. During the summer, drought continued during August and September. If drought decreased colonization, colonization may have reached a minimum in late September or early October. Similarly in early April, when the second field samples were collected, the seedlings may have not yet invested much carbon in root growth and AMF. Further growth during the spring could have led to an increase in colonization resulting in maximum colonization late in the spring. Based on these considerations, further work is needed to characterize annual changes in colonization.

One factor that is well known to affect AMF colonization is the level of phosphorus; high P concentrations tend to reduce colonization (Abbott and Robson 1991; Lingfei et al. 2005; Ryan et al. 2000; Smith et al. 2011). The two soils studied had different P content; the soil used in the mesocosm experiments was relatively poor in P with a content of $8.1 \mu\text{g.g}^{-1}$; while the soil at the field site had a P content of $43 \mu\text{g.g}^{-1}$. Nevertheless, even at this higher level, P can be a limiting factor for plant growth when soil moisture decreases. The decrease in moisture lengthens the pathway for water and nutrient movement to the root surface, thus limiting phosphorus uptake (Tinker and Nye 2000, Sardans et al. 2007). Hyphae of AMF extend as much as 10 cm beyond the root depletion zone and increase the rate and efficiency of nutrient transport over natural diffusion through the soil (Allen 2007; Smith and Read 1997). In terms of colonization, the two soils showed similar levels of natural colonization; and for the fall transplanting

experiment, inoculation caused similar increases in colonization. It is possible that adequate levels of P in the soil during the spring-field transplanting experiments may have led to low colonization rates. However, as discussed below, it is likely that other factors contributed to this phenomenon. Also, the effect of P on reducing AMF colonization is not universal (Johnson 2010). Plant ecotypes that have evolved in nutrient poor soils are often highly mycotrophic and show little ability to reduce colonization in response to high levels of nutrients (Azcon and Ocampo 1981; Hetrick et al. 1996; Johnson et al. 2008). Limited ability to reduce colonization in response to fertilization was also reported for *A. tridentata* ssp. *vaseyana* (Duke et al. 1994); arbuscular and total AMF colonization of plants supplemented with phosphate and nitrate were similar to those of control plants that did not receive nutrients. It would be interesting to investigate the colonization response to P fertilization with other subspecies of *A. tridentata* and with various AMF taxa. The lack of ability to restrict colonization would suggest that this process will be largely determined by the amount of AMF propagules present in the soil and the number of root tips, where infection occurs.

In contrast to my other experiments, plants in the field experiment started in the spring of 2012 showed no effect of inoculation on colonization after transplanting. One possible reason for this result was the level of colonization at the time of transplanting; which was only 24% for inoculated seedlings. This percent was lower than in all other experiments and may not have been sufficient to increase the AMF propagule density over the levels naturally occurring in the soil. In addition, low soil moisture may have negatively affected colonization. Burning of sagebrush habitats can increase soil water repellency, decreasing infiltration (Blank et al. 1995; de Jonge et al. 1999; DeByle 1973;

Robichaud and Hungerford 2000; Salih et al. 1973). Furthermore, the period between the time of transplanting and when the samples were collected for measurements of AMF colonization (May to July 2012) had lower than average precipitation. Low soil moisture due to poor infiltration and little precipitation may have favored the development of a deeper root system with less lateral roots and root tips in the upper layer of the soil, thus reducing the number of possible infection points. Low soil moisture may have also reduced extra-radical hyphal growth. Such effect was observed by Trent et al (1994b) in *A. tridentata* ssp *tridentata*; decreases in soil moisture reduced hyphal colonization and extra-radical hyphal density, but the effect was more drastic for the latter.

Evidence that low soil moisture can significantly reduce colonization was obtained in the mesocosm experiment started in the spring of 2012. Soil for this experiment was collected from the Big Foot Butte site, where the field experiment was conducted. However, the seedlings used for this experiment originated from a source population near Casper, Wyoming and were inoculated in cone-tainers rather than Jiffy pots and at the time of transplanting had high levels of colonization. For the 2012 mesocosm experiment, total colonization at the time of transplanting in April was 1.5 and 81% for non-inoculated and inoculated seedlings, respectively. Three months afterwards, differences in colonization were significant ($p = 0.007$). However, at this time the difference in colonization between treatments was much smaller, with a percent colonization of 9.9 and 24% for non-inoculated and inoculated seedlings, respectively. The large decrease in colonization observed in the inoculated seedlings suggests that low moisture limited the ability of the inoculum to infect and colonize the root system after transplanting.

One interesting result of the spring 2012 field experiment was that the percent colonization was lower in roots collected within the upper 20 cm of the soil (shallow roots) than in those collected below 30 cm (deep roots). Arbuscular mycorrhizal fungi typically occupy regions in the upper 50 cm of the soil profile, where nutrients and root length density are higher (Brundrett 1991; Smith 1978). Furthermore, various studies have shown that root colonization, extra-radical hyphal density, and spore abundance decrease with soil depth (An et al. 1990; Jakobsen and Erik Nielsen 1983; Kabir et al. 1997; Oehl et al. 2005; Rillig and Field 2003; Yang et al. 2010; Zajicek et al. 1986). This trend has also been observed in deep-rooted forbs, where colonization can occur to depths of 1 m (Zajicek et al. 1986). In contrast to these studies, I observed an increase in colonization with soil depth; the percent colonization was more than 3 fold higher in deep than shallow roots, 41.4 and 12.7%, respectively. Such differences may be attributed to the distribution of soil moisture. At the time of sampling, the soil was very dry in the upper 30 cm, but it was moist below 50 cm. The distribution of soil moisture appeared to have favored the development of deep tap roots (Abbott and Robson 1991), which sometimes reached more than 1 m. Given the soil moisture conditions, C export for root growth was probably directed to areas of the root system exposed to higher soil moisture. Under this scenario, AMF colonization would be higher in deep roots since infection occurs in areas of the root system that are actively growing (Allen 2001).

In mature plants of *A. tridentata*, hydraulic lift of deep soil water into shallow soil layers occurs at night and facilitates the maintenance of active roots near the soil surface (Dobrowolski et al. 1990; Richards and Caldwell 1987). Furthermore, hydraulic lift may enable prolonged root colonization and continued AMF activity including turgor

maintenance and exudation of water from hyphal tips to increase solubilization and absorption of nutrients (Allen 2006; Querejeta et al. 2003). The extent to which the young seedlings investigated in the spring 2012 field experiment experienced hydraulic lift is unknown. However, if this process occurred, it was not sufficient to support levels of colonization as high as those observed in the deep roots.

Increased levels of colonization associated with increased seedling survival

The results of my experiments indicate that increases in AMF colonization can increase survival of Wyoming big sagebrush seedlings. This was observed in the spring 2011 mesocosm experiment and in the field experiment started in the fall of 2012. In both cases, the increase in colonization was associated with an increase in survival of about 25%. In the two other experiments where survival was assessed, I did not see an increase in survival. However, in one of these experiments, no mortality occurred during the duration of the experiment; while in the other, inoculation did not increase AMF colonization after transplanting.

For the mesocosm experiments, the major cause of seedling mortality was summer drought. This conclusion is based on various observations. No mortality occurred in seedlings that were transplanted in the spring that received supplemental watering (well-watered treatment). In contrast, seedlings that did not receive additional watering experienced losses during mid and late summer. In addition, seedlings transplanted in early fall did not suffer any losses during the subsequent fall, winter, and spring where natural precipitation and temperatures were average for the region. Given that summer drought was the cause of seedling mortality, the effect of increasing AMF

colonization on improving survival appears to have been mediated by an increase in drought tolerance. Such effect is consistent with reports in other species that showed AMF induced improvements in seedling survival in arid environments or in response to artificially imposed drought (Abbaspour et al. 2012; Ouahmane et al. 2007; Requena et al. 2001).

Attempts were made to identify mechanisms by which AMF may have increased drought tolerance. Particularly in the spring 2011 mesocosm experiment, measurements of seedling growth and various other physiological parameters indicative of photosynthesis and transpiration were measured. When compared with well-watered seedlings, the seedlings that did not receive additional watering showed typical symptoms of water stress, including reduced growth, lower stomatal conductance, and higher water use efficiency. For these parameters, values for well-watered seedlings were significantly different from those of water-stressed seedlings independent of the inoculation treatment. In contrast, for net photosynthesis, NPQ, and F_s/F_o , the values of inoculated, water-stressed seedlings were intermediate to those of well-watered seedlings and non-inoculated, water-stressed ones. These results suggest that the increase in colonization partially ameliorated the effects of water stress, ultimately resulting in less mortality. This, however, does not appear to be related to an improvement in plant water status. Pre-dawn water potentials were measured at a time when significant losses of seedlings were occurring, but the values for non-inoculated and inoculated seedlings were similar indicating that AMF colonization is not positively correlated with drought avoidance. In Wyoming big sagebrush seedlings, the presence of AMF may however allow plants to tolerate soil water potentials that would be lethal to seedlings precluded from the AMF

symbiosis (Stahl et al. 1998). In these studies, non-inoculated and inoculated seedlings may have experienced similar decreases in water potential, but the effect of the decrease would be more damaging to plants with low rates of colonization.

The other experiment where an increase in survival was observed was in the field experiment started in the fall of 2012. In this experiment, the increase in colonization appeared to have increased tolerance to both cold and drought. Survival was analyzed separately between the time of transplanting and the end of winter (fall and winter survival), and between the end of winter and the end of the summer (spring and summer survival). Both of these analyses showed significant differences between non-inoculated and inoculated seedlings. For the fall-winter period, the majority of the losses occurred during the winter. These losses probably occurred in January, which was one of the coldest on record with temperatures dropping below -10°C for several days (NOAA/NCDC 2014). At the end of winter, survival in the non-inoculated, natural, and inoculated treatments was 51, 66, and 85%, respectively; and the differences were significant among the three treatments. These results contrast with those observed in the fall 2011 mesocosm experiment in which seedling mortality through the fall and winter was negligible for both non-inoculated and inoculated seedlings. A possible reason for the discrepancies between the field and mesocosm experiments is the severity of the winter, which was much colder in 2012-2013 than in 2011-2012.

AMF effects on cold tolerance have not received much attention, but a few studies have shown that AMF can increase tolerance to chilling and freezing temperatures (Latef and He 2011; Paradis et al. 1995; Zhou et al. 2012). The mechanisms responsible for these observations are not well understood, but may involve AMF-induced increases in

osmotic adjustment, antioxidant enzymes, and changes in membrane composition (Latef and He 2011). In addition, AMF effects on cold tolerance may reflect a more general effect of AMF on growth (Feddermann et al. 2010; Hardie and Leyton 1981; Smith and Smith 2011). In various species including a species within the *Artemisia* genus, the size and age of the seedlings have been positively related to their ability to develop cold hardiness (Hou and Romo 1998; Kozłowski and Pallardy 2002; Lim et al. 2014; Meyer and Badaruddin 2001). By promoting growth and/or higher photosynthetic capacity, AMF colonization may have increased the seedlings' ability to develop cold hardiness during the cold acclimation period, thus increasing their tolerance to frost (Korhonen et al. 2013).

In the field experiment started in the fall of 2012, inoculated seedlings also had higher survival than the other treatments during the spring and summer ($p < 0.05$). When only the survival that occurred during this period is considered, survival in the non-inoculated, natural, and inoculated treatments was 9.7, 16, and 34%, respectively. Thus, like in the spring 2011 mesocosm experiment, an increase in colonization was associated with an increase in survival. The reasons for this effect may be similar to those discussed above for the mesocosm experiment. In addition, the experiment in the field provided an environment where root and hyphal growth are much less limited by soil volume than in the mesocosm experiment. In the field, AMF colonization may have increased root biomass or altered root morphology to a larger extent than in the mesocosm experiment, leading to an improvement in water uptake. AMF-induced increases in root biomass have been reported, although this effect appears to be limited to plant species that are highly dependent on the symbiosis (Berta et al. 1993; Fan et al. 2011; Hetrick 1991; Sun and

Tang 2013; Tisserant et al. 1990). Colonization can also lead to changes in root morphology (Berta et al. 1993; Fan et al. 2011). In many species, P deficiencies promote a higher concentration of roots in the upper layers of the soil where P is more abundant (Ho et al. 2004). By improving P acquisition, AMF colonization may have reduced this effect, facilitating the development of roots in deep layers of the soil, where moisture was present. Clearly, the effect of AMF on root biomass and morphology of *A. tridendata* seedlings requires further investigation; such work may provide an insight into mechanisms by which AMF increased drought tolerance in my experiments.

Independent of the effect of AMF on seedling survival, comparison of the results from the two field experiments suggests that the factors causing mortality of seedlings transplanted in spring were somewhat different from those causing mortality of seedlings transplanted during the fall. For seedlings transplanted in spring, losses only occurred when temperatures were mild to warm and precipitation rare; thus strongly suggesting that the main factor causing seedling mortality was water stress. In contrast to the seedlings transplanted in the fall, none of the seedlings transplanted in spring died during the fall and winter despite the extreme cold temperatures experienced during the winter of 2013. Presumably, the advanced developmental stage of these seedlings allowed them to develop sufficient cold hardiness to withstand severe and repeated frosts (Hou and Romo 1998). In nature, sagebrush seeds germinate in early spring; those that survive through the summer have matured and grown before experiencing again cold temperatures. Freezing temperatures shortly after germination may cause losses of sagebrush seedlings (Lambrecht 2007). However, after surviving through the first

summer, my results suggest that cold is a less significant factor limiting sagebrush establishment.

In general, Wyoming big sagebrush seedlings that survive the first year in the field become established (Baker 2006; Meyer 1994). For the field experiment started in 2012, I did not observe such patterns. Significant losses of seedlings occurred during late spring and early summer of 2013. This may be attributed to very dry conditions. At the study site, precipitation during the spring and summer of 2013 was negligible. Taken together, the climatic conditions that the seedlings experienced during 2013 were likely to be responsible for the overall low rates of survival observed, which ranged from 4 to 31% depending on transplanting time and treatment. It seems likely that survival rates would be higher in a year experiencing average climatic conditions.

Effect of native-derived inoculum on the AMF community present in the seedlings

Prior to the comparison of AMF communities, the DNA sequences obtained were grouped based on phylogenetic analysis. This analysis revealed 6 phylotypes, which most likely underestimates the number of phylotypes present in the samples. The phylogenetic analysis was based on sequences from only one gene and there was poor resolution of some of the clades, particularly within *Glomus II* phylotype. Other studies have used phylotypes to estimate the diversity of AMF communities in various habitats through the amplification of the LSU-D2 region (Cesaro et al. 2008; Gollotte et al. 2004; Pivato et al. 2007; Rosendahl and Stukenbrock 2004; Torrecillas et al. 2012). Comparisons with these studies are complicated by differences in experimental approaches and the criteria employed in defining phylotypes (Liu et al. 2011; Redecker et al. 2013; Rosendahl and

Stukenbrock 2004). These studies have reported between 7 and 20 phylotypes, which is higher than the six we detected in sagebrush habitats. However, in some of these studies not all phylogenetic clusters were well supported by bootstrap values or certain sequences were left outside of a strongly supported phylotype, leading to an overestimation of their number when compared to my conservative approach (Gollotte et al. 2004; Rosendahl and Stukenbrock 2004; Torrecillas et al. 2012). Furthermore, the method used by Pivato et al. (2007), Cesaro et al. (2008), and Torrecillas et al. (2012) for amplification of the LSU region involved a seminested PCR using the LR1 and FLR4 primers in the second amplification. This primer pair is less specific for AMF than the FLR3 and FLR4 pair, but its use may allow the amplification of AMF sequences that are not amplified by FLR3 (Stockinger et al. 2010). When these factors are considered, the number of taxa in sagebrush habitats of southwestern Idaho appear to be similar to that reported for several mesic habitats (8-12) (Cesaro et al. 2008; Gollotte et al. 2004; Pivato et al. 2007; Rosendahl and Stukenbrock 2004), and somewhat lower than that reported for a semiarid area in Spain (20) (Torrecillas et al. 2012).

A recent study has characterized AMF taxa present in sagebrush seedlings collected from various habitats in southwestern Idaho (Carter et al. 2014). The taxa identified by Carter et al. (2014) show many similarities with the ones I identified, but also some differences. Most of the sequences that I obtained clustered within the phylotypes reported by Carter et al. (2014). One exception was, however, the *Glomus I* phylotype which was not identified by Carter et al. (2014). As analyzed by pairwise distances, my study also revealed more nucleotide sequence variation than that reported by Carter et al. (2014). Their study identified 16 OTUs with pairwise differences greater

than 6% between each other. Many of my sequences clustered within these OTUs, but I identified a larger number of OTUs, 29. This difference was likely attributed to the number of sequences that were analyzed, where I assessed approximately four times as many as the Carter et al. (2014) study. Another difference was in the relative abundance of certain phylotypes and OTUs. For example, sequences within the *Funneliformis* phylotype were relatively rare in the samples collected by Carter et al (2014), but they were found in more than 50% of the seedlings sampled. Similarly, in the Carter et al. study, the most abundant OTU was within the *Rhizophagus* phylotype. In contrast, the most common OTU identified was within the *Glomus II* phylotype. Interestingly, this OTU was found in 90% of the seedlings that I analyzed, but represented less than 5% of the sequences detected in field samples by Carter et al. (2014).

Various factors may account for the OTU differences mentioned above including site variations in AMF composition as well as soil disturbances associated with collecting soil and transplanting (Bell et al. 2009; Evans and Miller 1990; Husband et al. 2002; Schnoor et al. 2011; Sánchez-Castro et al. 2012). One of the sites analyzed by Carter et al. (2014), Kuna Butte, was the same site from which the soil for the mesocosm experiments was collected. As in other sites, the most common phylotype at Kuna Butte was *Rhizophagus*; which contrast with the results from my mesocosm experiments, where the *Glomus II* phylotype was the most common. One possible reason for this difference was the manner in which the samples were collected. Soil and sagebrush samples were collected from 20 random points throughout Kuna Butte by Carter et al. (2014), while I collected soil from mainly one area to minimize differences in soil texture. Also in my experiments, collection of the soil and filling of the pots most likely damaged

mycorrhizal networks. Furthermore, AMF networks may have been damaged during transplanting. Carter et al (2014) analyzed the AMF composition of seedlings that developed naturally in the field from seeds. These seeds presumably developed with minimal soil disturbances other than that caused by root penetration. Some AMF taxa may have low spore density and a high dependency on a hyphal network to maintain infectivity. For these taxa, the impact of soil disturbance on their ability to colonize roots would be more detrimental than in those that largely depend on spores for infection. Thus, differences in the abundance of certain taxa between the results reported by Carter et al. (2014) and myself may be partially attributed to damages incurred to fungal networks associated with collection of soil and transplanting (Schnoor et al. 2011).

Similar to the findings of Carter et al. (2014), all the taxa detected in my experiments were in the two Glomerales families, and the majority within the Glomeraceae. The reasons for only detecting taxa within this order are unclear, but they cannot be entirely attributed to the primers used. FLR3 and FLR4 discriminate against some species in the Diversisporales, Archaeosporales, and Paraglomerales (Mummey and Rillig 2007; Stockinger et al. 2010); however, they can amplify the LSU-D2 region of taxa in major families including Acaulosporaceae and Gigasporaceae (Gollotte et al. 2004; Mummey and Rillig 2007). Genera within the Glomerales often dominate arid and semiarid environments (Stutz et al. 2000; Torrecillas et al. 2012; Busby et al. 2013). Perhaps this dominance is more extreme in the steppes of southwestern Idaho. In addition, the more common phylotypes identified in my samples are known to produce small spores. Based on similar observations in other arid and semiarid regions, Stutz et al. (2000) suggested that some selection for taxa producing small spores occurs in these

regions. This could partly explain the scarcity of *Gigaspora* and *Scutellospora* taxa associated with my samples, which tend to develop large spores.

Native AMF were utilized in these experiments because they are better adapted to local climatic and edaphic conditions (Ji et al. 2010; Macek et al. 2011; Torrecillas et al. 2013) and may provide more benefits to native plants than exotic AMF (Pivato et al. 2007; Querejeta et al. 2006; Rakshit and Bhadoria 2008; Selosse et al. 2004). A concern when using native AMF is that multiplication in pot culture may alter the abundance of AMF phylotypes, resulting in an inoculum with an AMF composition different from that found in the field (Carter et al. 2014; Sykorova et al. 2007). These considerations are important when pre-inoculating seedlings because colonization by particular AMF taxa can influence subsequent colonization events (Mummey et al. 2009).

To identify possible changes in the AMF community induced by pre-inoculation, I compared the OTU richness, diversity, and composition of the AMF community of non-inoculated and inoculated seedlings. AMF OTU richness was not significantly altered by pre-inoculation. The richness values indicated that Wyoming big sagebrush seedlings were colonized simultaneously by 3 to 6 OTUs. Colonization by different AMF taxa may provide complementary benefits to an individual host (Jansa et al. 2008). Similar to the richness index, the Shannon diversity index did not differ significantly among inoculation treatments, except for the field experiment started in the spring of 2012. In this experiment, non-inoculated seedlings had a higher index of diversity than inoculated ones; although in absolute terms the difference was small, suggesting that slight differences may not be of biological significance. A more obvious difference in the diversity index was observed in the mesocosm experiment started in 2012. However, in

this experiment the difference was not between non-inoculated and inoculated seedlings, but rather between inoculated seedlings at the time of transplanting and that of seedlings collected three months after transplanting. Seedlings at the time of transplanting had a diversity index of 2.3, while those measured three months after transplanting had an average index between inoculated and non-inoculated seedlings of 1.18. Perhaps, the decrease in diversity was attributed to the dry conditions that the seedlings experience following transplanting.

In the five experiments conducted, I also analyzed whether the OTUs colonizing non-inoculated and inoculated seedlings were different using NMDS. None of these analyses revealed significant differences between non-inoculated and inoculated seedlings. Additionally, for the mesocosm experiment started in 2012, I compared the AMF community of pre-inoculated seedlings at the time of transplanting with that of seedlings collected 3 months later. No significant differences were observed between these samples. The reasons for the lack of a pre-inoculation effect on the AMF community are not clear. One possibility is that the AMF community was not altered during pot culture cultivation, although this seems unlikely based on other studies (Carter et al. 2014; Sykorova et al. 2007). Alternatively, if changes occurred, they were largely reversed during inoculation of sagebrush seedlings. A mechanism that could account for such reversal is the development of preferential associations between sagebrush and particular AMF taxa. Results reported by Busby et al (2013) suggest that in big sagebrush there is some selectivity during the formation of plant-AMF symbioses; in sites occupied by both big sagebrush and *Bromus tectorum* each plant species associated a distinct assemblage of AMF taxa. On the other hand, Carter et al (2014) found no significant

difference between the AMF phylotypes present in interspace soil and those in Wyoming big sagebrush seedlings, suggesting little AMF selection. However, the sites used in Carter et al (2014) were relatively pristine sagebrush habitats, which may host an AMF community that is more compatible with sagebrush plants.

Another factor that can alter the diversity and richness of AMF communities are seasonal changes in temperature and moisture. While several reports have documented these changes (Dumbrell et al. 2011; Li et al. 2010; Vandenkoornhuyse et al. 2002), many others have also indicated rather similar AMF communities throughout the seasons (Rosendahl and Stukenbrock 2004; Santos-González et al. 2007). The results of my study are in agreement with the latter. In both the mesocosm and field experiments, the AMF taxa colonizing Wyoming big sagebrush seedlings in mid or late summer were not significantly different from those colonizing the seedlings in early spring. Thus, independent of inoculation treatment and season of collection, the AMF community of sagebrush seedlings showed remarkable consistency. In addition, of the 29 OTUs detected, four were consistently dominant. The dominance of certain taxa may be a factor that contributes to reduced changes in AMF composition. This has been observed in grassland and semiarid environments, where the dominant AMF taxa did not vary temporally, while less common taxa varied in response to seasonal changes in soil moisture and concurrent changes in plant metabolism and phenology (Bever et al. 2001; Martínez-García et al. 2012; Pringle and Bever 2002; Ryan 2012; Sánchez-Castro et al. 2012). Although these are possible explanations for my results, further work is needed to determine the extent to which differences in OTU abundance reflect differences in OTU

frequencies within the soil or the potential influence of preference by sagebrush seedlings.

Conclusions

The results of this study indicate that pre-inoculation of Wyoming big sagebrush seedlings with native AMF can increase colonization after transplanting. These results were determined from soils collected at only two sites and therefore it is difficult to predict whether similar effects will be observed in other sagebrush habitats. However, the natural levels of colonization that I identified were similar to those reported by others, suggesting that inoculation could increase colonization in other sagebrush communities. Under conditions where drought or cold temperatures caused seedling losses, the increase in colonization resulted in an increase in seedling survival. Many questions remain, however, about the mechanisms by which AMF colonization increased sagebrush survival. Based on other studies, this may be attributed to the ability of AMF-colonized plants to withstand lower water potentials than non-AMF plants or due to AMF-induced changes in root biomass or architecture that allow the seedlings to increase water uptake (Hetrick 1991; Stahl et al. 1998). Mesocosm studies comparing non-AMF versus AMF seedlings may provide a stark contrast that can better address and test these questions. One important concern when using AMF inoculum is that this treatment may reduce AMF diversity or alter the AMF composition (Mummey et al. 2009; Schwartz et al. 2006). The results of my study indicate that the native AMF community was rather resilient to changes in composition during multiplication and subsequent inoculation. Consequently, this approach appears to be a feasible strategy for increasing AMF colonization of sagebrush seedlings without the risks associated with changes to the AMF

community. The dominance of a few taxa may also simplify the production of inoculum. Production of monospecific cultures of these taxa and subsequent mixing would allow the development of a more precise and uniform inoculum. Development of monospecific cultures could elucidate the consequences of colonization by individual AMF taxa, thus developing inoculum that optimizes benefits to Wyoming big sagebrush seedlings. However, some questions remain concerning the effects of soil disturbance to the AMF community caused by transplanting and the reasons for the predominance of certain OTUs.

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APPENDIX A

Tables

Table 1. Total biomass and shoot over root ratio of inoculated and non-inoculated sagebrush seedlings transplanted on May 6, 2011. Mean ($\pm$ SE) of three to five replications. Means not labeled by the same letter are statistically significant according to the Tukey HSD test ($p < 0.05$).

Treatment	July Sampling		October Sampling	
	Total Dry Weight (g)	Shoot:Root	Total Dry Weight (g)	Shoot:Root
Non-Inoculated + Water			5.89 $\pm$ 0.65 ^a	1.58 $\pm$ 0.12 ^a
Inoculated + Water			8.56 $\pm$ 1.53 ^a	1.72 $\pm$ 0.58 ^a
Non-Inoculated	1.04 $\pm$ 0.15 ^a	1.90 $\pm$ 0.30 ^a	2.06 $\pm$ 0.66 ^b	1.58 $\pm$ 0.15 ^a
Inoculated	1.23 $\pm$ 0.30 ^a	2.01 $\pm$ 0.28 ^a	1.45 $\pm$ 0.71 ^b	1.96 $\pm$ 0.41 ^a

Table 2. Test for significance of time, treatment, and time x treatment on chlorophyll fluorescence and gas exchange parameters of sagebrush seedlings in the spring mesocosm experiment.

Parameter	Time			Treatment			Time x Treatment		
	DF	F value	<i>p</i> value	DF	F value	<i>p</i> value	DF	F value	<i>p</i> value
F_v/F_m	6	1.702	0.131	2	0.877	0.420	11	2.039	0.035
$\Delta F/F_m'$	6	2.706	0.01917	2	0.172	0.84245	11	2.493	0.00956
<i>NPQ</i>	6	2.937	0.0122	2	4.403	0.0153	11	1.626	0.1071
F_s/F_o	6	1.227	0.30113	2	5.647	0.00508	11	1.496	0.14949
Net Photosynthesis	6	8.155	1.02e-06	2	7.318	0.00129	11	1.741	0.08180
Stomatal conductance	6	9.123	2.15e-07	2	16.442	1.36e-06	11	1.399	0.193
Water use efficiency	6	6.253	2.87e-05	2	5.872	0.00444	11	0.659	0.77172

Table 3. Total biomass, shoot to root ratio, and foliar carbon isotope ratios ($\delta^{13}\text{C}$) of non-inoculated and inoculated sagebrush seedlings transplanted on October 1, 2011. Mean ($\pm$ SE) of 8 seedlings measured seven and half months after transplanting. Within a column, means labeled by the same letter are not statistically significant.

Treatment	Total dry weight (g)	Shoot to root ratio	Leaf $\delta^{13}\text{C}$ (‰)
Non-inoculated	3.29 $\pm$ 0.481 ^a	3.16 $\pm$ 0.276 ^a	-28.644 ($\pm$ 0.35) ^a
Inoculated	3.54 $\pm$ 0.605 ^a	3.12 $\pm$ 0.379 ^a	-28.920 ($\pm$ 0.51) ^a

Table 4. Percent total mycorrhizal and arbuscular colonization at the time of field transplanting in spring and fall 2012. Mean ($\pm$ SE) of 4 to 6 seedlings. Within a column, means labeled with different letters are significantly different ($p < 0.05$) based on Tukey-Kramer least square means test.

Treatment	Spring 2012		Fall 2012	
	Total colonization	Arbuscular colonization	Total colonization	Arbuscular colonization
Non-inoculated	0.5 ($\pm$ 0.3) ^c	0 ^c	0.8 ($\pm$ 0.6) ^c	0 ^b
Natural (inoculated with soil from the Big Foot site)	15.2 ($\pm$ 3.3) ^b	2.0 ($\pm$ 1.2) ^b	12.8 ($\pm$ 2.2) ^b	2.3 ($\pm$ 1.1) ^{ab}
Inoculated (inoculated with soil from the pot cultures)	24.2 ($\pm$ 3.5) ^a	8.3 ($\pm$ 3.6) ^a	34.5 ($\pm$ 8.5) ^a	6.1 ($\pm$ 2.4) ^a

Table 5. Richness (S) and Shannon-Wiener Diversity (H') indices of AMF OTU's isolated from the roots of (n) individual sagebrush seedlings (Mean $\pm$ SE) for each study. Within a particular experiment, values that differ significantly from the non-inoculated seedlings are indicated by an asterisk. For the spring 2012 experiment, sequences were also analyzed just before transplanting, pre-transplanted treatment. Total Diversity represents the diversity of pooled samples.

	Experiment	Treatment	n	Richness (S)	Diversity (H')	Total Diversity
Mesocosm	Spring 2011	Non-inoculated	5	4.70 ± 1.20	1.21 ± 0.32	1.87
		Inoculated	5	5.00 ± 0.58	1.13 ± 0.09	1.57
	Fall 2011	Non-inoculated	8	4.13 ± 0.72	0.95 ± 0.22	1.52
		Inoculated	8	3.40 ± 0.42	0.74 ± 0.14	1.80
	Spring 2012	Pre-transplant	6	6.00 ± 0.89	2.30 ± 0.59 *	2.10
		Non-inoculated	8	4.75 ± 0.49	1.28 ± 0.11	1.97
		Inoculated	8	4.43 ± 0.57	1.08 ± 0.13	1.71
Field	Spring 2012	Non-inoculated	6	5.00 ± 0.52	0.96 ± 0.10	1.21
		Inoculated	6	3.83 ± 0.31	0.64 ± 0.07 *	0.76
	Fall 2012	Non-inoculated	6	3.67 ± 0.56	0.82 ± 0.15	1.05
		Inoculated	6	2.83 ± 0.48	0.59 ± 0.17	0.83

APPENDIX B

Figures

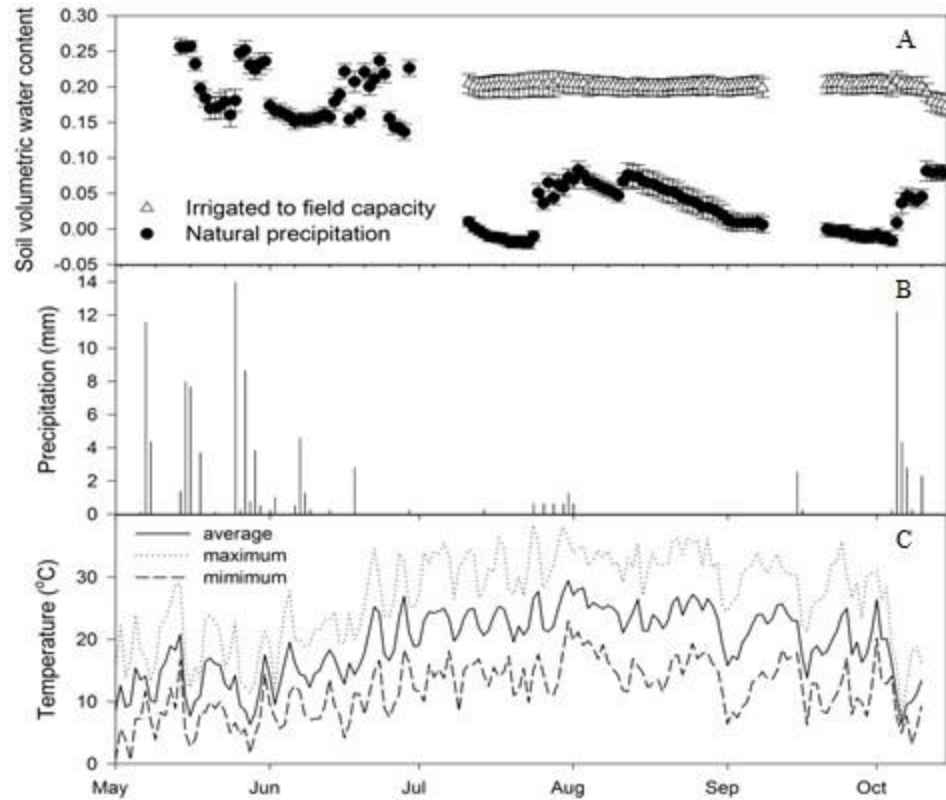


Figure 1. Environmental conditions that *Artemisia tridentata* seedlings experienced from May 6 to October 15, 2011. A, Soil volumetric water content (volume of water to volume of soil). B, Daily precipitation (mm). C, Daily average, minimum, and maximum temperatures (°C).

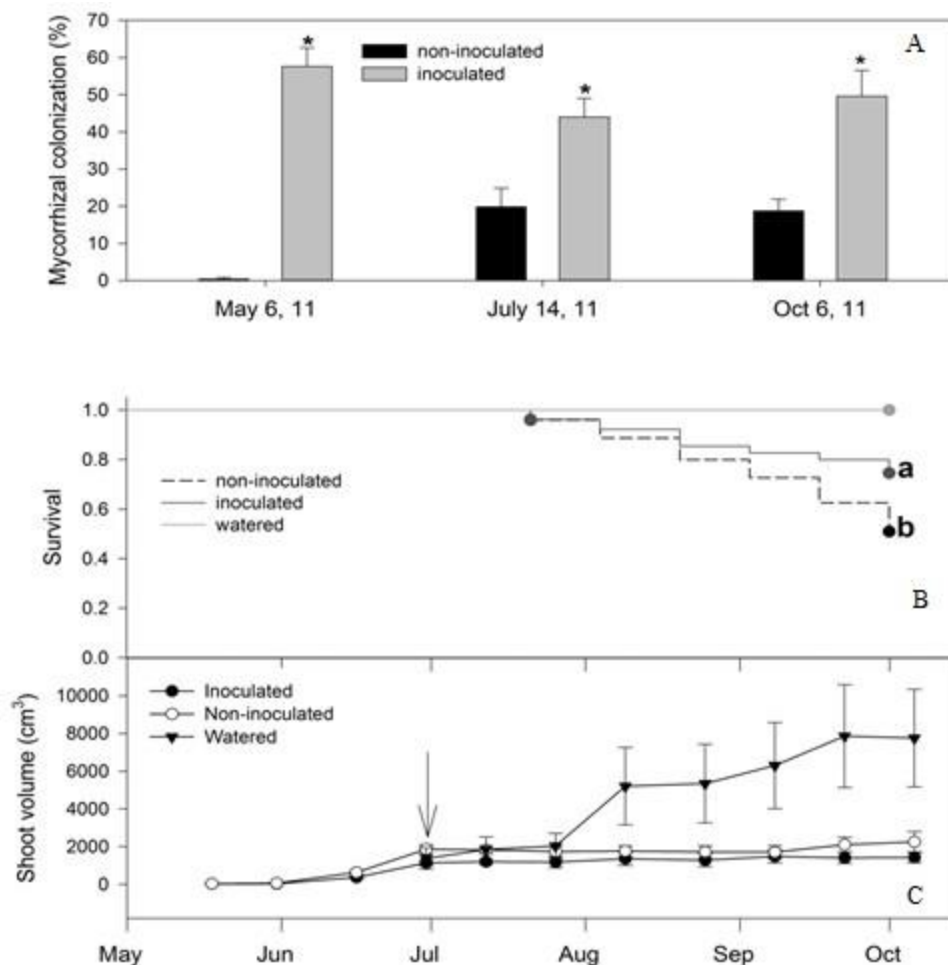


Figure 2. Mycorrhizal colonization (A), survival (B), and shoot growth (C) of sagebrush seedlings growing in pots at the Idaho Botanical Gardens. A, Mycorrhizal colonization at the time of transplanting (May 6) and two and five months afterwards. Each bar represents the mean ($\pm$ SE) of at least five seedlings. For a particular date, bars marked by an asterisk are significantly higher than the non-inoculated seedlings ($p < 0.05$) based on an independent t -test. B, Fraction of surviving seedlings over the course of the experiment; different letters indicate significant differences ($p < 0.05$) based on a log-rank test. C, Shoot volume, each symbol represents the mean ($\pm$ SE) of all living seedlings in a particular treatment. The arrow, on July 1, indicates the initiation of irrigation for the watered treatment.

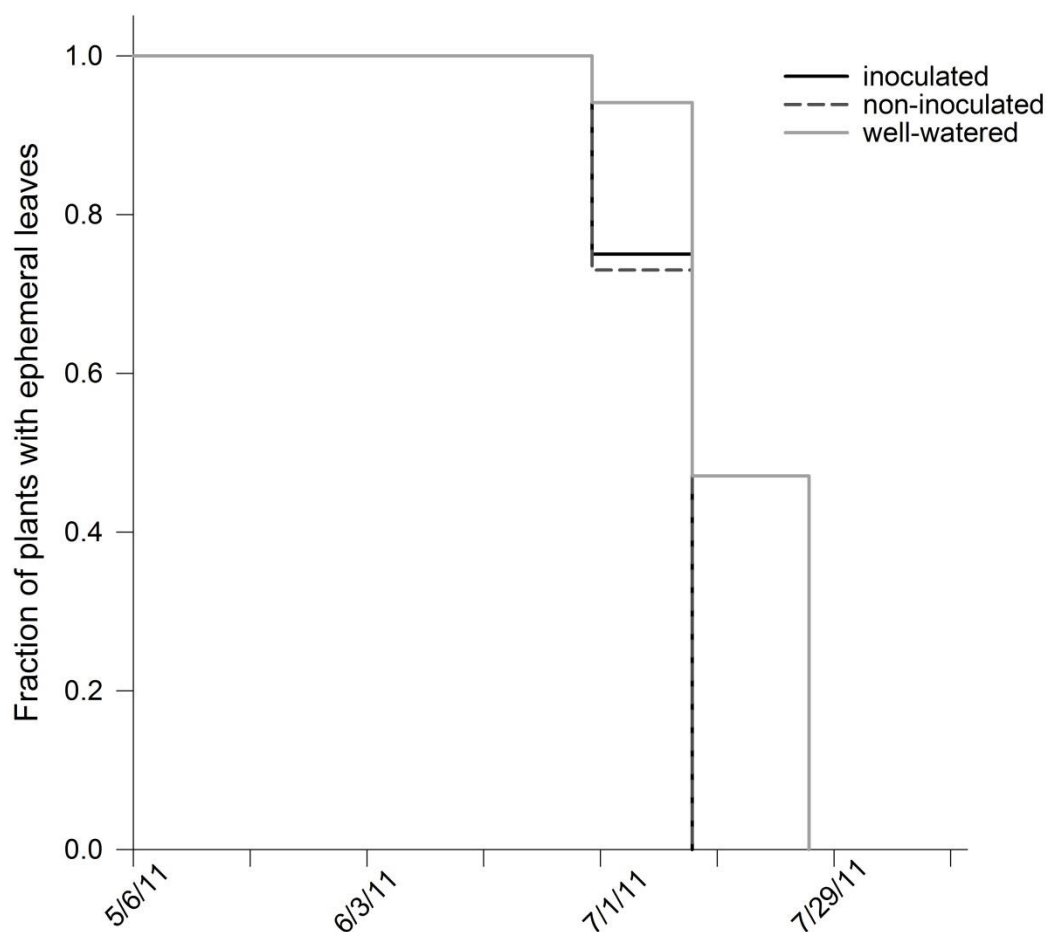


Figure 3. Time course of ephemeral leaf senescence as measured by the fraction of plants with ephemeral leaves. Based on a log-rank test, drop of ephemeral leaves occurred later in well-watered plants than those experiencing natural precipitation ($p < 0.001$).

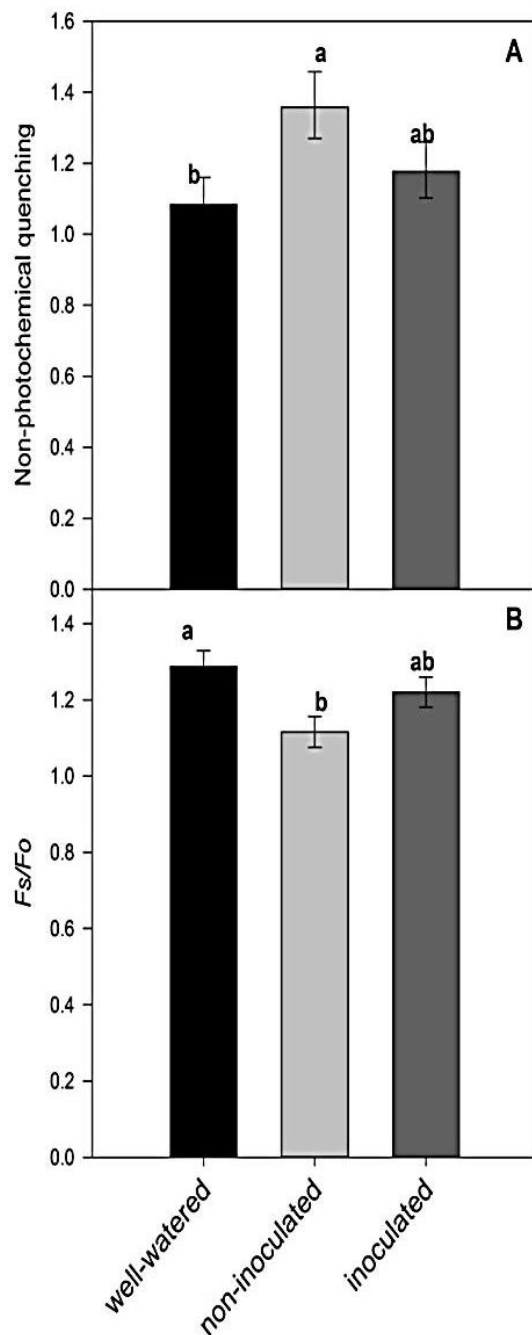


Figure 4. Chorophyll fluorescence parameters of *Artemisia tridentata* seedlings grown outdoors during the spring and summer of 2011. Non-photochemical quenching (A) and steady state fluorescence normalized to its dark-adapted state (F_s/F_o) (B) of well watered seedlings and of non-inoculated and inoculated seedlings receiving only natural precipitation. Each bar represents the mean (+SE) of 35 measurements. Measurements were made biweekly from 6/29/11 to 9/22/11 and 5 measurements were conducted for each measuring date and treatment. Means not labelled with the same letter are significantly different ($p < 0.05$) based on Tukey-Kramer least square mean test.

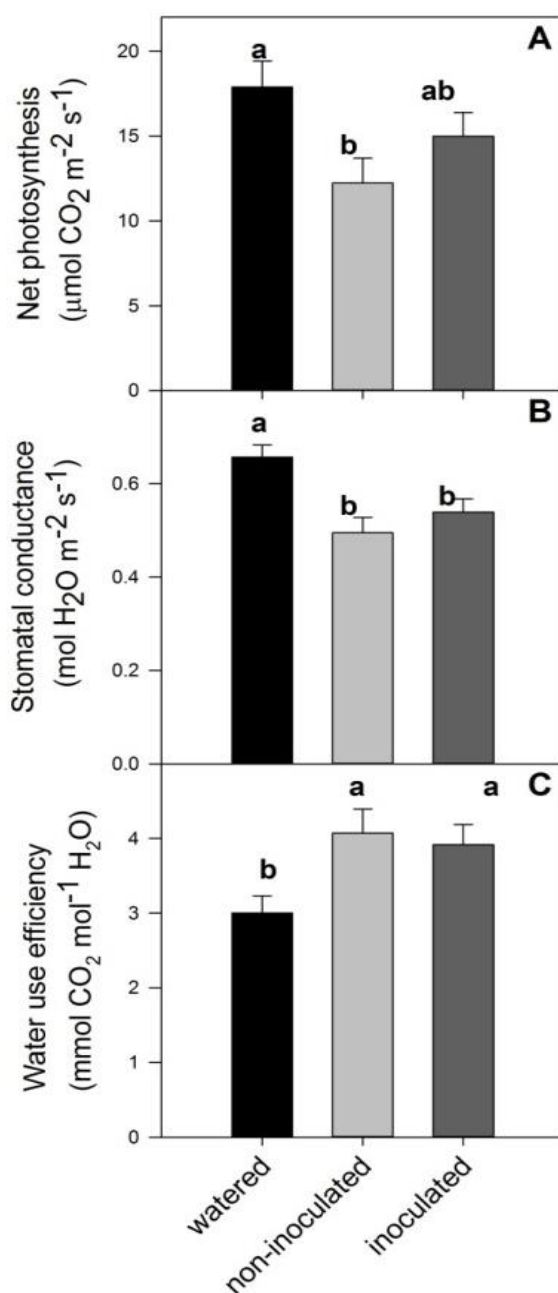


Figure 5. Influence of watering and mycorrhizal inoculation on gas exchange parameters of *Artemisia tridentata* seedlings grown outdoors during the spring and summer of 2011. Net photosynthesis (A), stomatal conductance (B), and photosynthetic water use efficiency (C) of well watered seedlings and of non-inoculated and inoculated seedlings that only received natural precipitation. Each bar represents the mean (+SE) of 35 measurements; measurements were made biweekly from 6/29/11 to 9/22/11 and 5 measurements were conducted for each measuring date and treatment. Means not labelled with the same letter are significantly different ($p < 0.05$) based on Tukey-Kramer least square mean test.

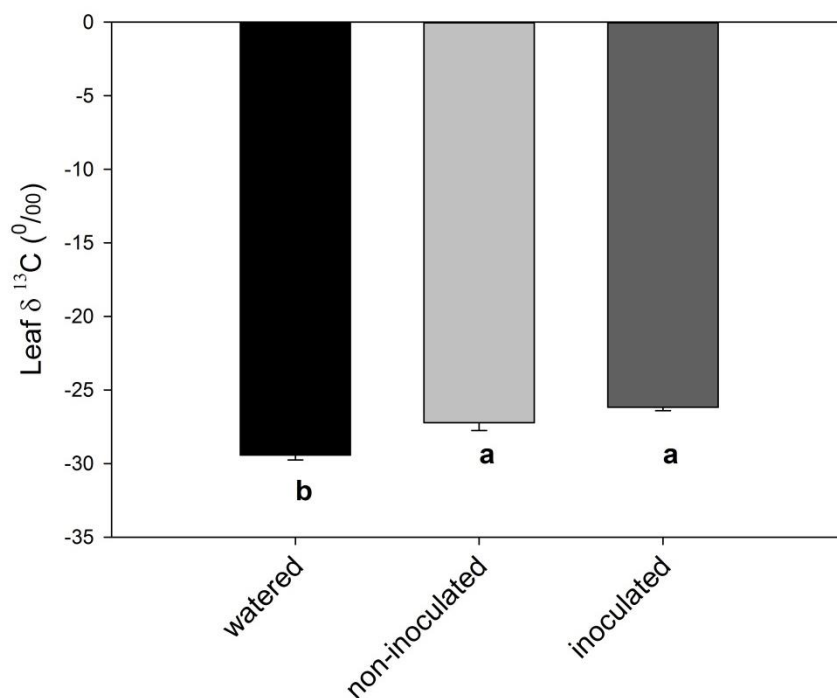


Figure 6. Foliar carbon isotope ratios ($\delta^{13}\text{C}$) of *Artemisia tridentata* seedlings. Each bar represents the mean ($\pm$ SE) of 5 seedlings. Leaves for $\delta^{13}\text{C}$ analysis were collected on October 6, 2011, at the end of spring transplanting experiment. Means not labelled with the same letter are significantly different ($p < 0.05$) based on Tukey-Kramer least square mean test.

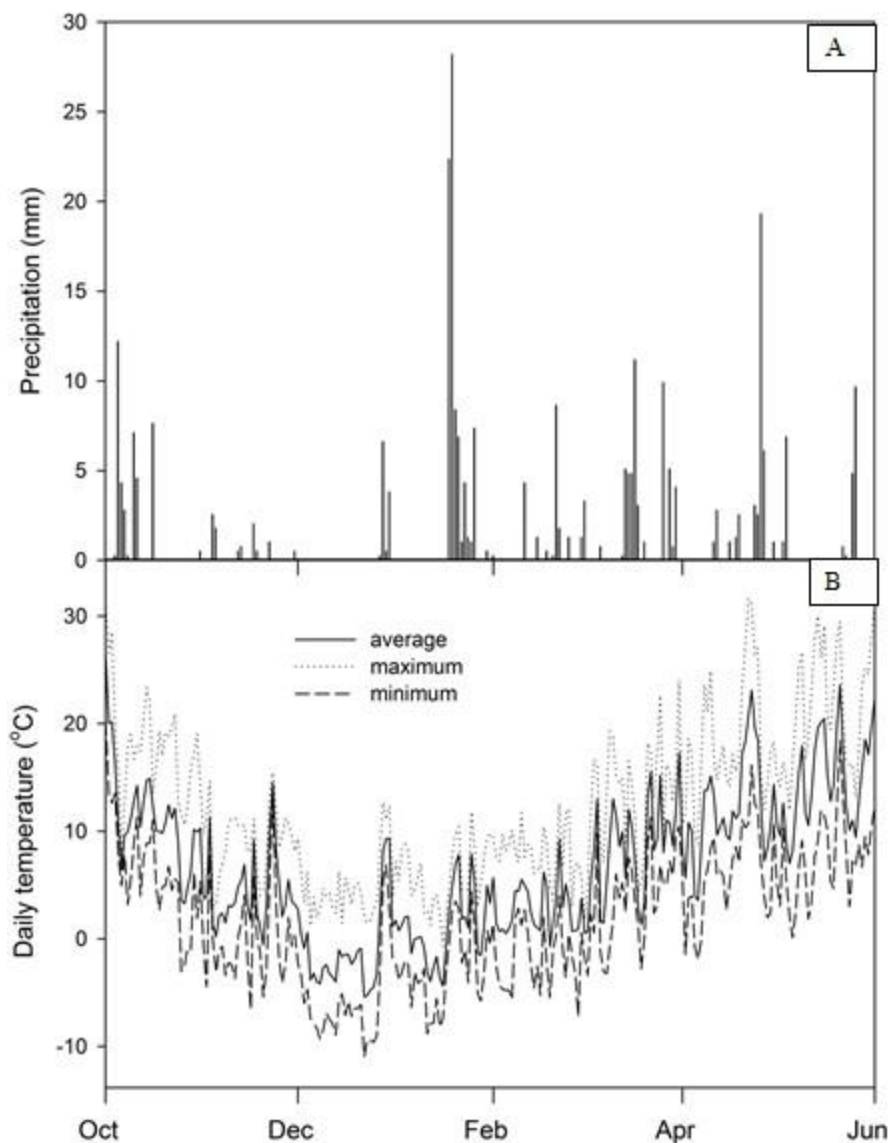


Figure 7. Weather conditions from October 1, 2011 to mid- June, 2012 at the Idaho Botanical Gardens experienced by seedlings in the fall mesocosm experiment. A, daily precipitation; B, Daily average, minimum, and maximum temperatures.

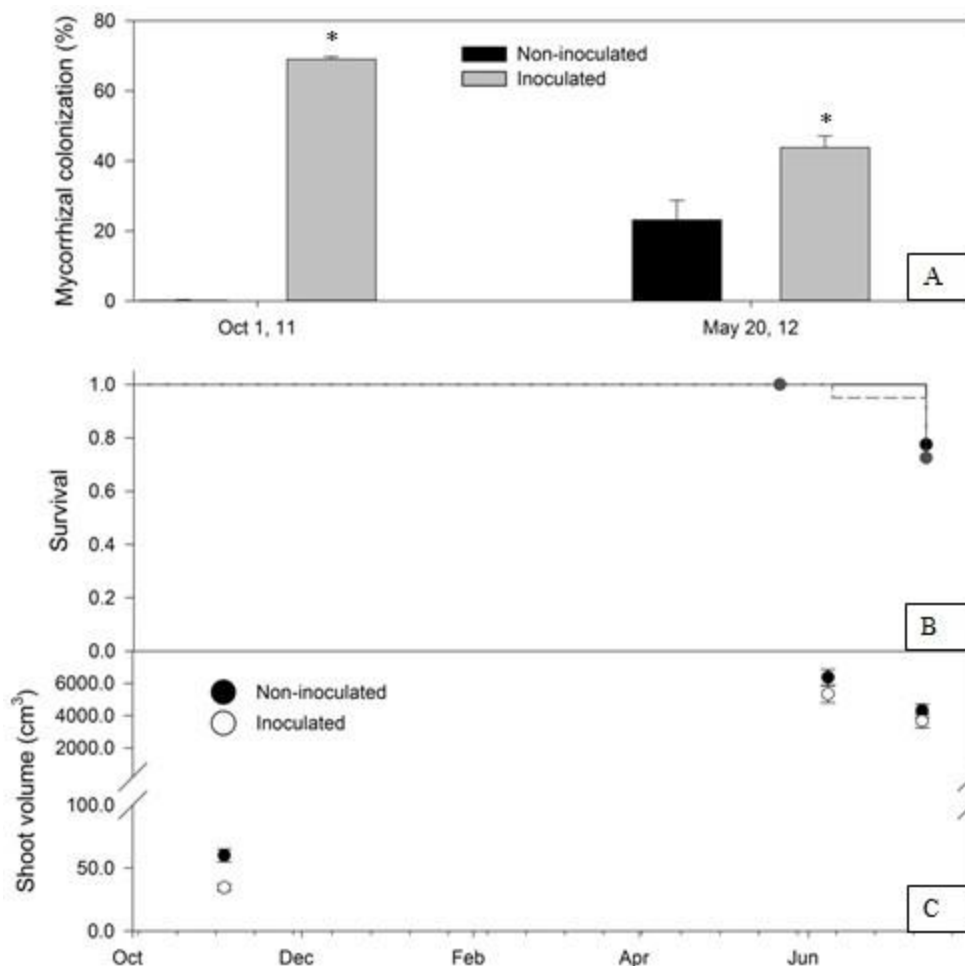


Figure 8. Mycorrhizal colonization (A), survival (B), and shoot growth (C) of sagebrush seedlings growing in pots at the Idaho Botanical Gardens from October 2011 to June 2012. A, Mycorrhizal colonization at the time of transplanting and seven and half months later. Each bar represents the mean ($\pm$ SE) of 8 seedlings. For a particular date, bars marked by an asterisk are significantly higher than the non-inoculated seedlings ($p < 0.05$) based on an independent t -test. B, Fraction of surviving seedlings over the course of the experiment. C, Shoot volume, each symbol represents the mean ($\pm$ SE) of all living seedlings in a particular treatment measured one month after transplanting (November 3, 2011), at the height of vegetative growth (June 8, 2011), and after the senescence of ephemeral leaves (July 12, 2012). Inoculation did not significantly affect seedling survival or shoot volume.

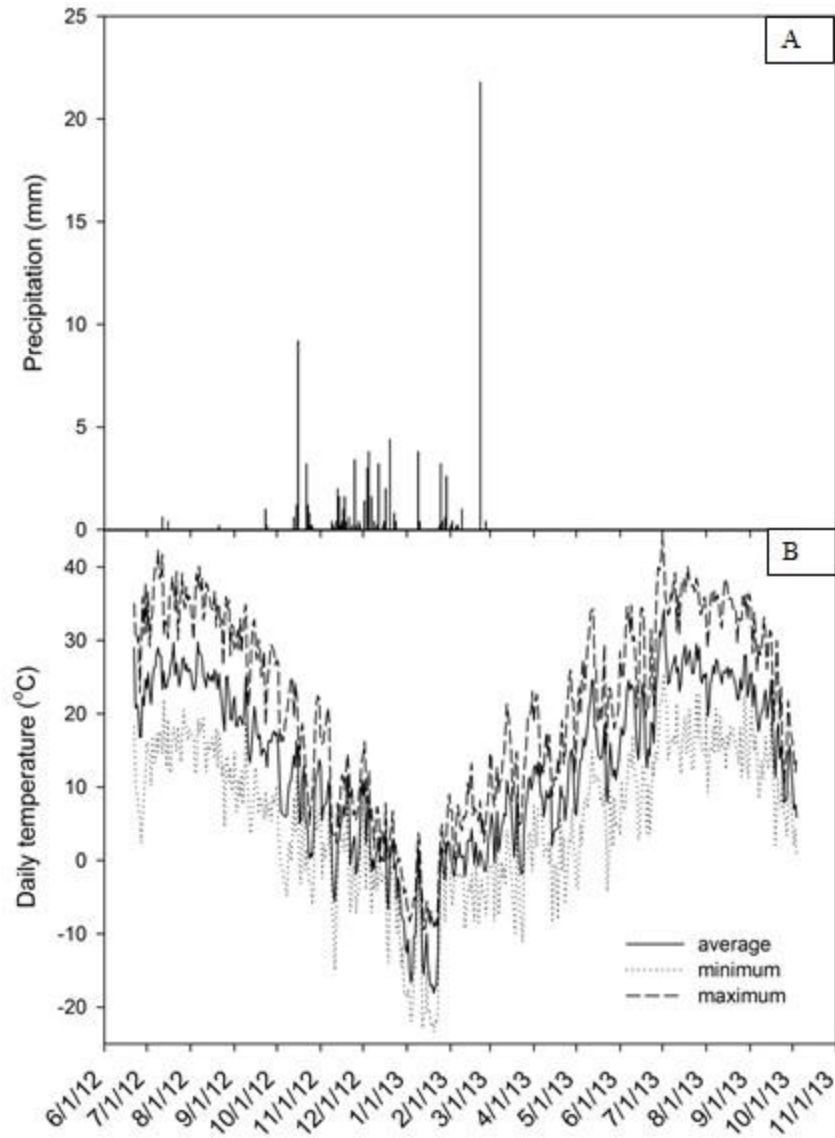


Figure 9. Weather conditions from June 1, 2012 to November 1, 2013 at the field site near Big Foot Butte, Snake River Birds of Prey, NCA. Panel (A) presents naturally occurring precipitation (mm) over the duration of the spring and fall 2012 field experiments. Panel (B): Temperature (°C) is depicted showing daily average, minimum, and maximum values.

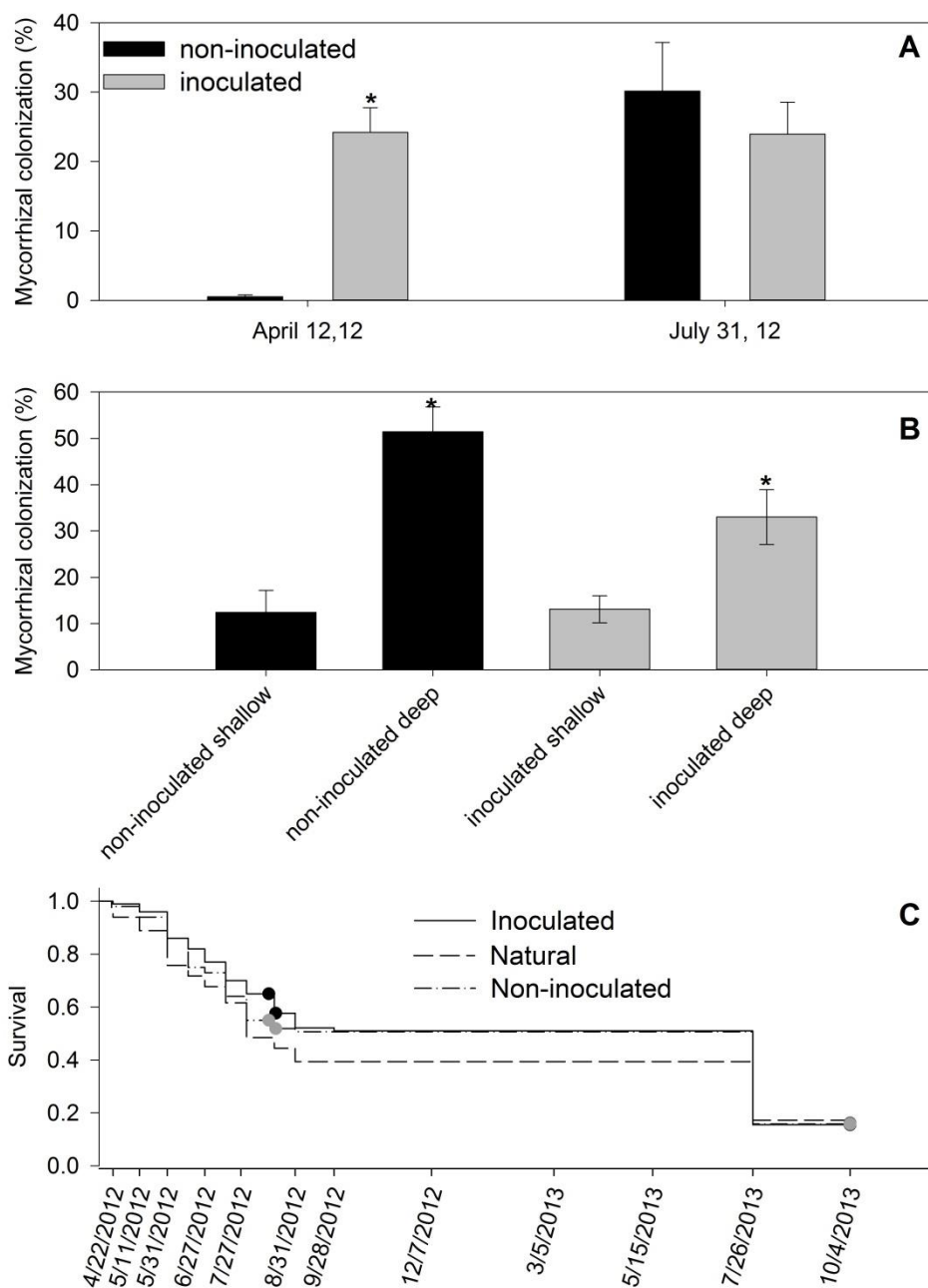


Figure 10. Mycorrhizal colonization by treatment (A) and rooting depth (B), and survival (C) of sagebrush seedlings transplanted into the Big Foot Butte site on April 2012. Each bar represents the mean ($\pm$ SE) of six seedlings. In A, bars marked by an asterisk are significantly different from the non-inoculated seedlings ($p < 0.05$). In B, shallow roots were collected within the top 20 cm of the soil and deep roots below 30 cm; bars marked by an asterisk are significantly different from the shallow roots. C, Proportion of live seedlings over the course of the experiment. Based on a log-rank test, survival was similar among treatments; x-axis in C indicates dates when survival was measured.

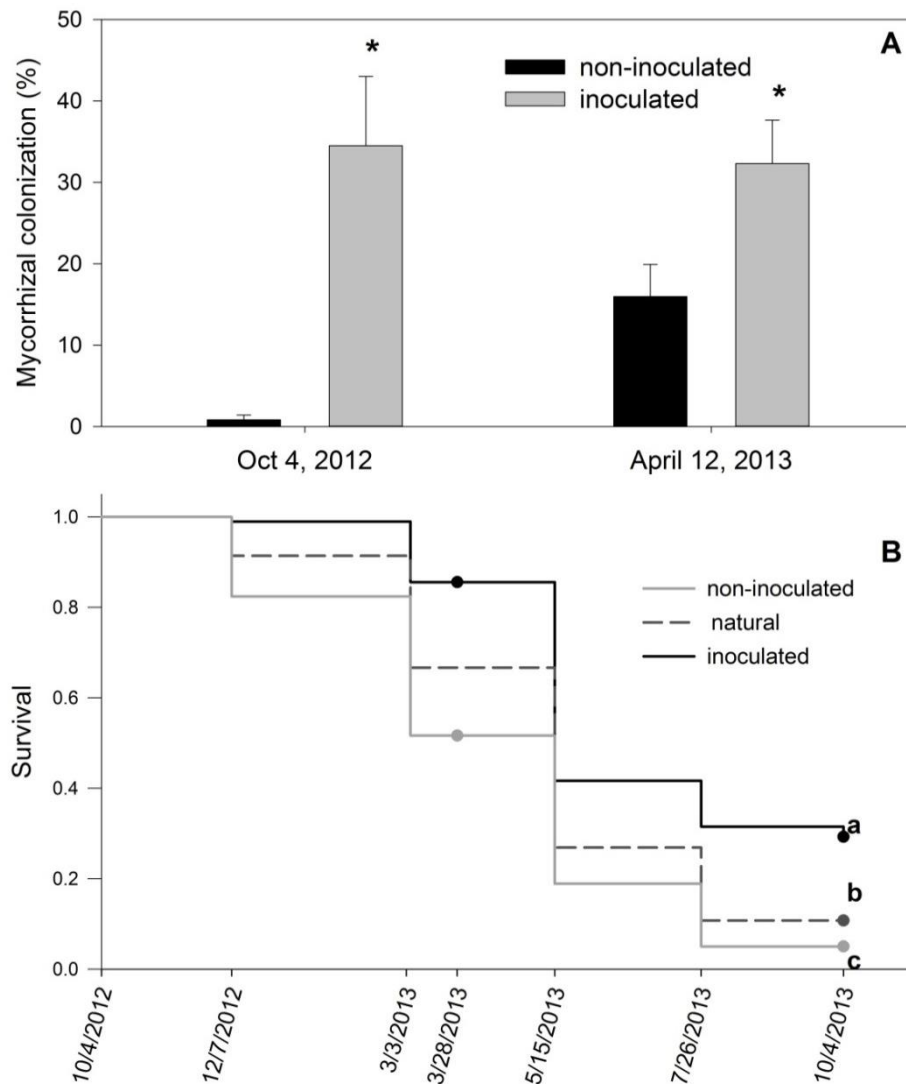


Figure 11. Mycorrhizal colonization (A) and survival (B) of sagebrush seedlings transplanted into the Big Foot Butte site on October 4, 2012. In A, each bar represents the mean ($\pm$ SE) of six seedlings; bars marked by an asterisk are significantly different from the non-inoculated seedlings ($p < 0.05$). B, Proportion of live seedlings over the course of the experiment; different letters indicate significant differences based on a log-rank test.

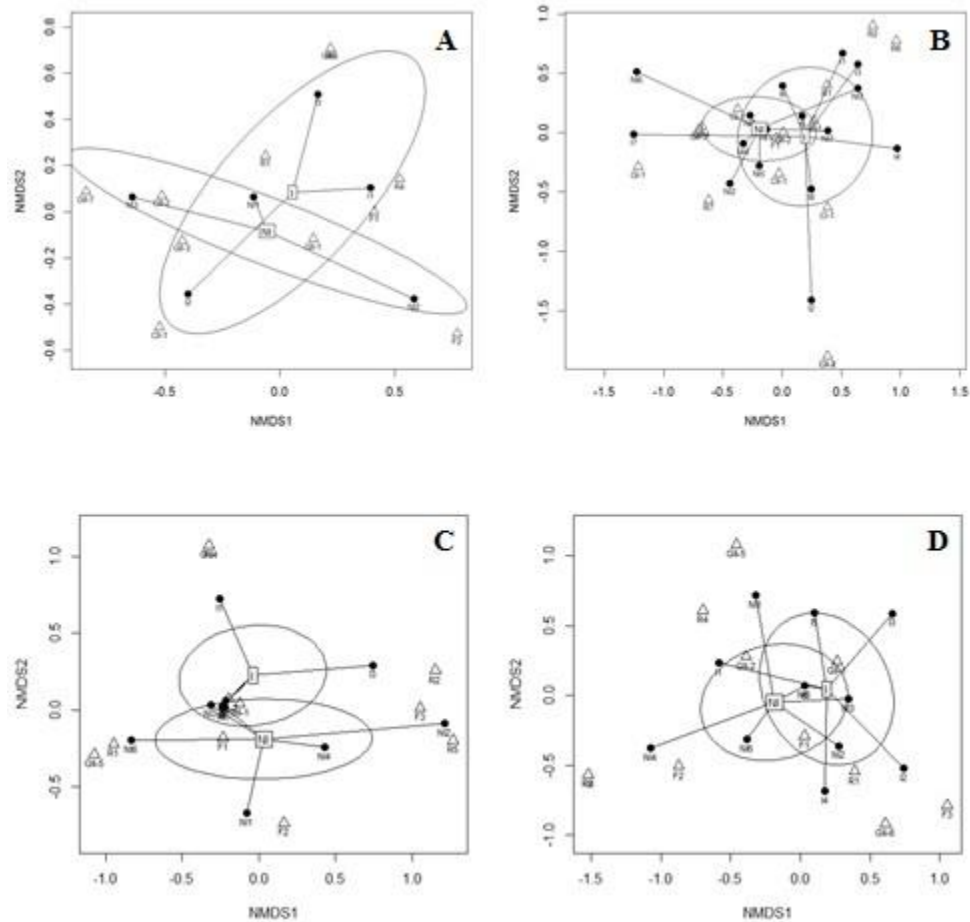


Figure 13. Comparison of arbuscular mycorrhizae communities of non-inoculated and inoculated seedlings by nonmetric multidimensional scaling. Samples of *Artemisia tridentata* seedlings were collected several months after transplanting in the spring 11 (A) and fall 11 (B) mesocosm experiments, and the spring 12 (C), and fall 12 (D) field experiments. OTUs are represented by open triangles and individual seedlings with filled circles. The centroids for the each treatment are labeled (NI, non-inoculated seedlings; I, inoculated seedlings) and linked to the samples that relate to them. Ellipses represent 95% confident limits around the centroids of each variable. OTUs are labeled based on their phylotype (CI, *Claroideoglomus I*; CII, *Claroideoglomus II*; F, *Funneliformis*; GI, *Glomus I*; GII, *Glomus II*; R, *Rhizophagus*) and OTU number within its phylotype.

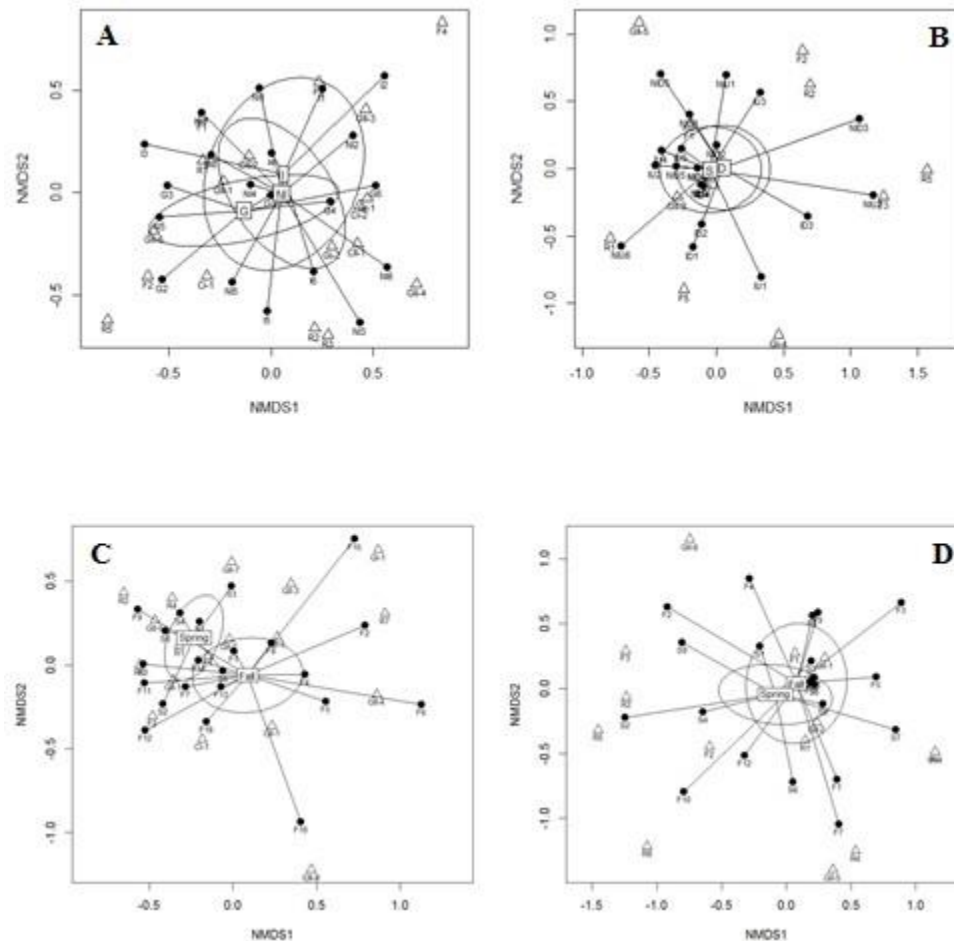


Figure 14. Analysis of arbuscular mycorrhizae communities by nonmetric multidimensional scaling. A, Comparison of AMF communities obtained from greenhouse-inoculated seedlings prior to transplanting (G) with those obtained from non-inoculated (NI) and inoculated (I) seedlings three months after transplanting in the 2012 mesocosm experiment. B, Analysis of AMF communities obtained from shallow (S) and deep (D) roots in the spring 2012 field experiment. C, Comparison of AMF communities in seedlings collected 5 and 8 months after the spring and fall 2011 mesocosm experiments, respectively. D, Comparison of AMF communities in seedlings collected 4 and 8 months after the spring and fall 2012 field experiments, respectively. OTUs are represented by open triangles and individual seedlings with filled circles. The centroids for each categorical variable are labeled and linked to the samples that relate to them. Ellipses represent 95% confident limits around the centroids of each variable.

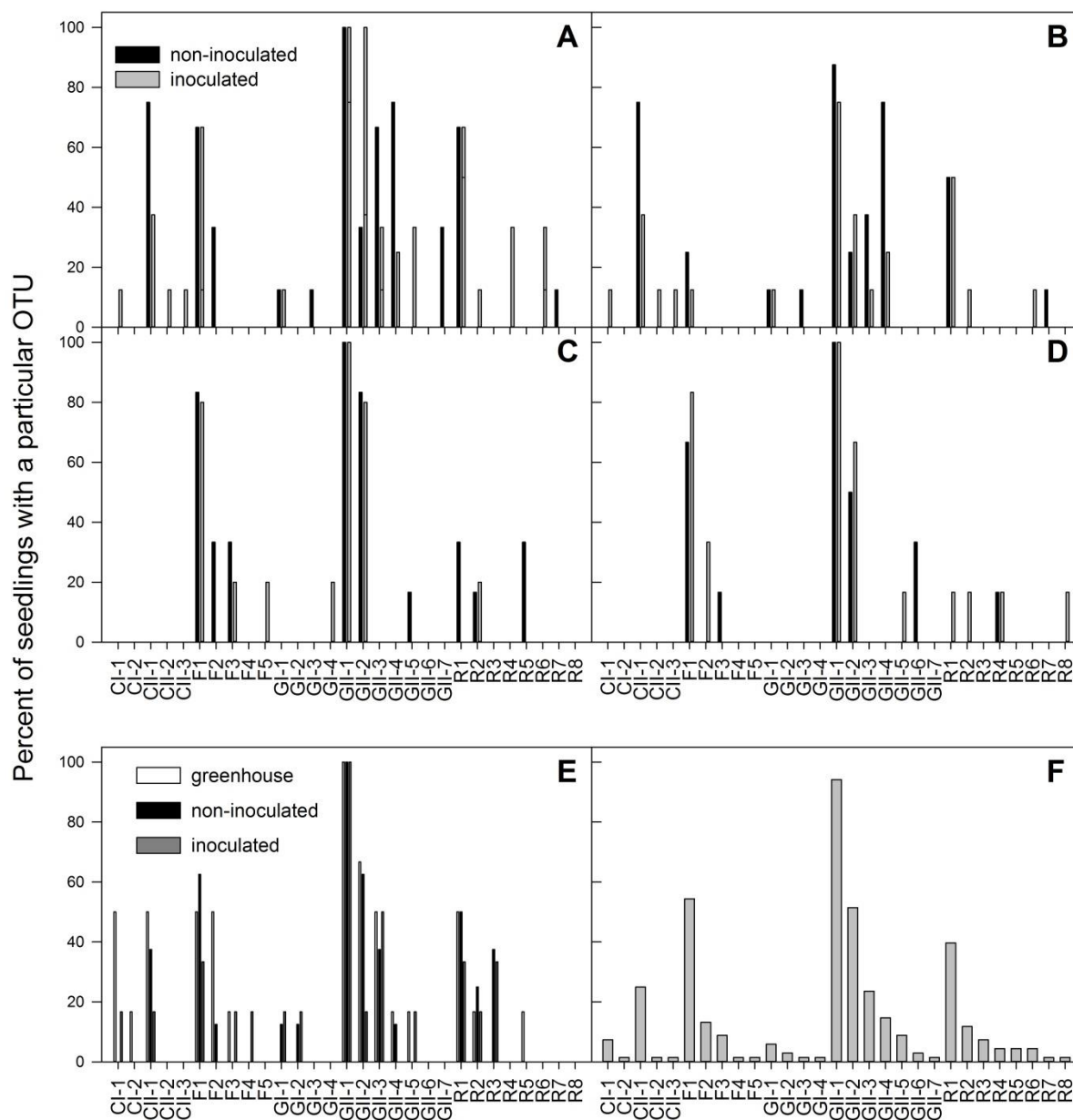


Figure 15. Frequency of different AMF OTUs found to be colonizing roots of *Artemisia tridentata* seedlings. Results from the spring 11 (A), fall 11 (B) and spring 12 (E) mesocosm experiments, and from the spring 12 (C) and fall 12 (D) field experiments. F, Overall frequencies observed after combining the results from all the seedlings analyzed. In A to E, non-inoculated and inoculated seedlings were collected several months after transplanting. In E, greenhouse seedlings refer to inoculated seedlings that were analyzed just prior to transplanting.