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

by

Bill Eugene Davidson

A thesis

submitted in partial fulfillment

of the requirements for the degree of

Master of Science in Biology

Boise State University

May 2015

© 2015

Bill Eugene Davidson

ALL RIGHTS RESERVED

BOISE STATE UNIVERSITY GRADUATE COLLEGE

DEFENSE COMMITTEE AND FINAL READING APPROVALS

of the thesis submitted by

Bill Eugene Davidson

Thesis Title: Consequences of pre-inoculation with native arbuscular mycorrhizae on root colonization and survival of Wyoming big sagebrush (*Artemisia tridentata* ssp. *wyomingensis*) seedlings after transplanting

Date of Final Oral Examination: 04 March 2015

The following individuals read and discussed the thesis submitted by student Bill Eugene Davidson, and they evaluated his presentation and response to questions during the final oral examination. They found that the student passed the final oral examination.

Marcelo D. Serpe, Ph.D. Chair, Supervisory Committee

Stephen J. Novak, Ph.D. Member, Supervisory Committee

Merlin M. White, Ph.D. Member, Supervisory Committee

The final reading approval of the thesis was granted by Marcelo D. Serpe, Ph.D., Chair of the Supervisory Committee. The thesis was approved for the Graduate College by John R. Pelton, Ph.D., Dean of the Graduate College.

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.

ACKNOWLEDGEMENTS

I would like to acknowledge, first and foremost, my primary advisor, Dr. Marcelo Serpe. Marcelo provided both support and guidance while allowing for my independence. His knowledge and patience are greatly appreciated. I would elaborate further on my immense gratitude and respect for this man if not for his continual prompting to be concise in my writing. Marcelo, thank you, sincerely, for your mentorship!

My committee has also had a significant impact on my understanding and appreciation for the complexities of biological and ecological systems, for this I must acknowledge them. Dr. Stephen Novak, your eccentric genius and witty skepticism never fails to stimulate serious contemplation and insight into the dynamic processes of evolution. Dr. Merlin White, your passion for discovery and reverence for cryptic organisms have elucidated entire dimensions of biodiversity, emphasizing the complex and interdependent nature of existence.

I acknowledge the Department of Biological Sciences of Boise State University for their commitment to science and education as well as the supportive community of faculty and students that I have found here. I would like to express my appreciation to Dr. Jim Smith, whose generosity and knowledge of phylogenetics resulted in the addition of an invaluable component to my research. I must acknowledge the contributions of several undergraduate research assistants who spent countless hours in the field, on the microscope, and in the hood to make this work possible. Thank you, Eric Roberts,

Russell Holten, Rachel Barron, and Craig Carpenter for all your time and effort. Additionally, thanks to Tom Dixon, Emma Wilson, Jay Zimmers, Rob Spaul, Laura Wendell, Kevin Glueckert, Chris Porterfield, and many others for providing friendship and encouragement. I would like to acknowledge Judy Lonsdale, who taught and demonstrated the subtle nuances of effective teaching and for helping me realize that no amount of personal appreciation for biology and ecology will promote conservation without also educating the populous.

This research project and my graduate education would not have been possible without funding support. I was fortunate to serve as a Teaching Assistant during the course of my graduate education and I am thankful for this opportunity and the students that helped me to develop skills in public outreach and education. Funding was contributed through grants from the United States Department of Agriculture through the National Institute for Food and Agriculture (NIFA) as well as the Great Basin Native Plant Project (GBNPP). Funding was also contributed by the United States Department of the Interior through the Bureau of Land Management, Great Basin Restoration Initiative (GBRI). Additional resources were contributed by the Idaho Department of Fish and Game and the many volunteers that they helped to recruit. Several individuals from these agencies provided personal and professional support that helped see this thesis to completion and deserve special recognition, namely: Anne Halford, Michael Young, Dr. Nancy Shaw, Matt Fisk, Erin Denney, Jan Gurr, and Alexis Malcomb. The efforts of these organizations and individuals are greatly appreciated and I am honored to have the opportunity to contribute to our shared goal of restoring and maintaining our natural resources.

I owe a wealth of gratitude to my family for their patience, love, support, and understanding. My loving wife, Merry, and children, Tré and Rain, deserve praise and thanks for more than I can mention. Paul and Michal Davidson, Heather Davidson, Kira Burrup, Mikell Hite, Dalen and Liam Ashley, Derek Yee, and David Richardson continue to play pivotal roles in my life and I cannot express my love and respect enough for these individuals. Last, though certainly not least, I am grateful for my mother, Linda, for giving me life (for not taking my life away) and for encouraging me to pursue my passions with tenacity.

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 *a* 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 38
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 91
Tables 91
APPENDIX B 97
Figures 97

LIST OF TABLES

Table 1. Total biomass and shoot-to-root ratio of inoculated and non-inoculated sagebrush seedlings transplanted on May 6, 2011. Mean (± SE) of three to five replications. Means not labeled by the same letter are statistically significant according to the Tukey HSD test ($p < 0.05$). 92

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. 93

Table 3. Total biomass, shoot-to-root ratio, and foliar carbon isotope ratios ($\delta^{13}C$) of non-inoculated and inoculated sagebrush seedlings transplanted on October 1, 2011. Mean (± SE) of 8 seedlings measured seven and half months after transplanting. Within a column, means labeled by the same letter are not statistically significant......................... 94

Table 4. Percent total mycorrhizal and arbuscular colonization at the time of field transplanting in spring and fall 2012. Mean (±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. 95

Table 5. Richness (*S*) and Shannon-Wiener Diversity (*H'*) indices of AMF OTU's isolated from the roots of (n) individual sagebrush seedlings (Mean ± 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. 96

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 (ºC)...... 98

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 (± 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 (± SE) of all living seedlings in a particular treatment. The arrow, on July 1, indicates the initiation of irrigation for the watered treatment.. 99

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 ($p < 0.001$)....................................... 100

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 (*Fs/Fo*) (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. 101

Figure 5. Influence of watering and mycorrhizal inoculation on gas exchange parameters of *Artemisia tridentanta* 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. 102

Figure 6. Foliar carbon isotope ratios ($\delta^{13}C$) of *Artermisia tridentata* seedlings. Each bar represents the mean (-SE) of 5 seedlings. Leaves for $\delta^{13}C$ 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. 103

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. 104

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 (± 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 (± 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........ 105

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. 106

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 (± 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. 107

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 (± 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. 108

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.. 109

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.. 110

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 mesoscosm 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. 111

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. 112

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
$\delta^{13}C$	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 (Augspurger 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 mychorrizal 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 (Finlay 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 was 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 *Antenarria 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 km^2 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 (Muscha 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 (Prevéy 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. 1994). 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 tridendata* 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. 1994). For these shrubs (Trent et al. 1994), 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 ± 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 $\mu g\ g^{-1}$ 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 $^{13}C/^{12}C$ isotope ratio ($\delta\, ^{13}C$).

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 2012. 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 $\mu g\ g^{-1}$ 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°18'48.43"N, 116°21'48.57"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 πab^2, where a and b are half the height and width, respectively). For the determination of the shoot-to-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, Optisciences, Inc, NH). After dark adaptation, minimal fluorescence (F_0) was measured with a red light of 0.1 µmol $m^{-2}s^{-1}$ and maximal fluorescence (F_m) following a pulse of saturating light of 8000 µmol $m^{-2}s^{-1}$ and 0.8 second duration. The samples were subsequently exposed to actinic light of 600 µmol $m^{-2}s^{-1}$ 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_o, 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. 2002). 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 CO_2 concentration of 400 µmol mol^{-1}, 50 (± 5) % relative humidity, ambient temperature, and a saturating light intensity of 1700 µmol m^{-2} s^{-1}. The infrared gas analyzer was matched prior to each measurement and values of net photosynthesis and transpiration were recorded after the CO_2 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 CO_2 assimilation and transpiration rates per unit area of each sample. From the values of CO_2 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 ($\delta^{13}C$). 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 ≥ 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 $\delta^{13}C$ 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 log-rank 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 ($H = -\Sigma_{i=1}^{S} p_i \ln p_i$, 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 (± 7.08) % and an arbuscular colonization of 6.7 (± 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 (± 3.36) and 43.99 (± 6.28) %, respectively. Similarly, arbuscular colonization was lower in non-inoculated than inoculated seedlings, 2.99 (± 0.78) and 10.2 (± 3.95) %, respectively. On October 6, 2011, when the Spring 2011 experiment was ended, difference in total colonization were maintained; 18.74 (± 1.69) and 49.59 (± 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 (± 0.52) and 16.7 (± 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 (± 5.1) and 25.3 (± 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 MPa (± 0.06) for the well-watered seedlings, -2.76 MPa (± 0.49) for the non-inoculated seedlings, and -3.08 MPa (± 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 *Fs/Fo* 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 *Fs/Fo*; 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 ($\delta^{13}C$) were consistent with the results of WUE obtained using the portable gas exchange system. The well-watered plants had significantly lower $\delta^{13}C$ 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 colonizaiton 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 $\delta^{13}C$ (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 cm^3 per month, while those transplanted in fall had a rate of about 750 cm^3 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 $\delta^{13}C$ 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 $\delta^{13}C$ of -26.76 (± 0.34), while those transplanted in fall had $\delta^{13}C$ of -

28.77 (± 0.29). Differences in both the shoot-to-root ratio and $\delta^{13}C$ 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 (14.2ºC). 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 30ºC. 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 -9.1ºC. 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 5.3ºC in March to an average of 20.3ºC for June. July and August average temperatures were 27 and 24.8ºC, 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 (± 2.8) and 41.4 (± 4.8) %, respectively. Differences were also noted for arbuscular colonization, although these values were very low 0.5 (± 0.2) and 2.1 (± 1.1) % for shallow and deep roots, respectively ($p = 0.02$).

For samples collected on July 31-2012, analysis of foliar $\delta^{13}C$ revealed no differences between treatments. Non-inoculated and inoculated seedlings had values of -27.51 (± 0.27) and -27.06 (± 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 $\delta^{13}C$ were collected on May 20, 2013. No differences were observed between treatments. Non-inoculated and inoculated seedlings had values of -28.40 (± 0.22) and -28.22 (± 0.32) ‰, respectively. These values of foliar $\delta^{13}C$ 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 $\delta^{13}C$ of -27.29 (± 0.28), while those transplanted in fall had $\delta^{13}C$ of -28.40 (± 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 phylogentic 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 (Fig. 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 (Friese 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. 1994). Overall, percent colonization was higher in spp. *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. 1994). 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. (1994) 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 $\mu g.g^{-1}$; while the soil at the field site had a P content of 43 $\mu g.g^{-1}$. 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. (1994) 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 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 than 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; Kozlowski 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 mesoscom 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. 2012b). 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. 2012b). Furthermore, the method used by Pivato et al. (2007), Cesaro et al. (2008), and Torrecillas et al. (2012b) 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. 2012b).

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. 2012a; Torrecillas et al. 2012b; 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. 2010b; 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.

LITERATURE CITED

Abbaspour H, Saeidi-Sar S, Afshari H, Abdel-Wahhab MA (2012) Tolerance of Mycorrhiza infected Pistachio (Pistacia vera L.) seedling to drought stress under glasshouse conditions. Journal of Plant Physiology 169: 704-709. doi: 10.1016/j.jplph.2012.01.014.

Abbott L, Robson A (1991) Factors influencing the occurrence of vesicular-arbuscular mycorrhizas. Agriculture, ecosystems & environment 35: 121-150.

Al-Karaki GN, Clark RB (1999) Mycorrhizal influence on protein and lipid of durum wheat grown at different soil phosphorus levels. Mycorrhiza 9: 97-101. doi: 10.1007/s005720050293.

Aldridge CL, Boyce MS (2007) Linking occurrence and fitness to persistence: Habitat-based approach for endangered Greater Sage-Grouse. Ecological Applications 17: 508-526. doi: 10.1890/05-1871.

Alguacil M, Torres M, Torrecillas E, Díaz G, Roldán A (2011) Plant type differently promote the arbuscular mycorrhizal fungi biodiversity in the rhizosphere after revegetation of a degraded, semiarid land. Soil Biology and Biochemistry 43: 167-173.

Allen EB, Rincon E, Allen MF, Pérez-Jimenez A, Huante P (1998) Disturbance and Seasonal Dynamics of Mycorrhizae in a Tropical Deciduous Forest in Mexico1. Biotropica 30: 261-274.

Allen MF (1983) Formation of vesicular-arbuscular mycorrhizae in Atriplex gardneri (Chenopodiaceae): seasonal response in a cold desert. Mycologia: 773-776.

Allen MF (2001) Modeling arbuscular mycorrhizal infection: is % infection an appropriate variable? Mycorrhiza 10: 255-258. doi: 10.1007/s005720000081.

Allen MF (2006) Water dynamics of mycorrhizas in arid soils. Fungi in biogeochemical cycles Cambridge Univ Press, New York: 74-97.

Allen MF (2007) Mycorrhizal fungi: Highways for water and nutrients in arid soils. Vadose Zone Journal 6: 291-297. doi: 10.2136/vzj2006.0068.

Allen MF, Smith WK, Moore TS, Christensen M (1981) Comparative Water Relations And Photosynthesis Of Mycorrhizal And Non-Mycorrhizal Bouteloua-Gracilis Hbk Lag Ex Steud. New Phytologist 88: 683-693. doi: 10.1111/j.1469-8137.1981.tb01745.x.

An Z-Q, Grove J, Hendrix J, Hershman D, Henson G (1990) Vertical distribution of endogonaceous mycorrhizal fungi associated with soybean, as affected by soil fumigation. Soil Biology and Biochemistry 22: 715-719.

Anderson JE, Inouye RS (2001) Landscape-scale changes in plant species abundance and biodiversity of a sagebrush steppe over 45 years. Ecological monographs 71: 531-556.

Appoloni S, Lekberg Y, Tercek MT, Zabinski CA, Redecker D (2008) Molecular Community Analysis of Arbuscular Mycorrhizal Fungi in Roots of Geothermal Soils in Yellowstone National Park (USA). Microbial Ecology 56: 649-659. doi: 10.1007/s00248-008-9384-9.

Augé RM (2001) Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11: 3-42. doi: 10.1007/s005720100097.

Augé RM (2004) Arbuscular mycorrhizae and soil/plant water relations. Canadian Journal of Soil Science 84: 373-381.

Augé RM, Moore JL, Cho KH, Stutz JC, Sylvia DM, Al-Agely AK, Saxon AM (2003) Relating foliar dehydration tolerance of mycorrhizal Phaseolus vulgaris to soil and root colonization by hyphae. Journal of Plant Physiology 160: 1147-1156. doi: 10.1078/0176-1617-01154.

Augé RM, Toler HD, Moore JL, Cho K, Saxton AM (2007) Comparing contributions of soil versus root colonization to variations in stomatal behavior and soil drying in

mycorrhizal< i> Sorghum bicolor</i> and< i> Cucurbita pepo</i>. Journal of plant physiology 164: 1289-1299.

Augé RM, Toler HD, Saxton AM (2014) Arbuscular mycorrhizal symbiosis alters stomatal conductance of host plants more under drought than under amply watered conditions: a meta-analysis. Mycorrhiza: 1-12.

Augspurger CK (1984) Seedling survival of tropical tree species: interactions of dispersal distance, light-gaps, and pathogens. Ecology 65: 1705-1712.

Azcon R, Ocampo J (1981) Factors affecting the vesicular-arbuscular infection and mycorrhizal dependency of thirteen wheat cultivars. New Phytologist 87: 677-685.

Baker NR (2008) Chlorophyll fluorescence: A probe of photosynthesis in vivo. Annual Review of Plant Biology 59: 89-113. doi: 10.1146/annurev.arplant.59.032607.092759.

Baker WL (2006) Fire and restoration of sagebrush ecosystems. Wildlife Society Bulletin 34: 177-185.

Barzana G, Aroca R, Antonio Paz J, Chaumont F, Carmen Martinez-Ballesta M, Carvajal M, Manuel Ruiz-Lozano J (2012) Arbuscular mycorrhizal symbiosis increases relative apoplastic water flow in roots of the host plant under both well-watered and drought stress conditions. Annals of Botany 109: 1009-1017. doi: 10.1093/aob/mcs007.

Barzana G, Aroca R, Bienert GP, Chaumont F, Manuel Ruiz-Lozano J (2014) New Insights into the Regulation of Aquaporins by the Arbuscular Mycorrhizal Symbiosis in Maize Plants Under Drought Stress and Possible Implications for Plant Performance. Molecular Plant-Microbe Interactions 27: 349-363. doi: 10.1094/mpmi-09-13-0268-r.

Bell CW, Acosta-Martinez V, McIntyre NE, Cox S, Tissue DT, Zak JC (2009) Linking Microbial Community Structure and Function to Seasonal Differences in Soil Moisture and Temperature in a Chihuahuan Desert Grassland. Microbial Ecology 58: 827-842. doi: 10.1007/s00248-009-9529-5.

Belnap J (2003) The world at your feet: desert biological soil crusts. Frontiers in Ecology and the Environment 1: 181-189.

Berta G, Fusconi A, Trotta A (1993) VA mycorrhizal infection and the morphology and function of root systems. Environmental and Experimental Botany 33: 159-173. doi: 10.1016/0098-8472(93)90063-l.

Bever JD, Schultz PA, Pringle A, Morton JB (2001) Arbuscular Mycorrhizal Fungi: More Diverse than Meets the Eye, and the Ecological Tale of Why The high diversity of ecologically distinct species of arbuscular mycorrhizal fungi within a single community has broad implications for plant ecology. Bioscience 51: 923-931.

Blank R, Young J, Allen F (1995) The soil beneath shrubs before and after wildfire: implications for revegetation.

Bonfante P, Perotto S (1995) Tansley Review No. 82 Strategies of arbuscular mycorrhizal fungi when infecting host plants. New Phytologist: 3-21.

Brooks ML, D'Antonio CM, Richardson DM, Grace JB, Keeley JE, DiTomaso JM, Hobbs RJ, Pellant M, Pyke D (2004) Effects of invasive alien plants on fire regimes. BioScience 54: 677-688.

Brundrett MC (1991) Mycorrhizas in natural ecosystems. Academic Press.

Busby RR, Gebhart DL, Stromberger ME, Meiman PJ, Paschke MW (2011) Early seral plant species' interactions with an arbuscular mycorrhizal fungi community are highly variable. Applied Soil Ecology 48: 257-262. doi: 10.1016/j.apsoil.2011.04.014.

Busby RR, Stromberger ME, Rodriguez G, Gebhart DL, Paschke MW (2013) Arbuscular mycorrhizal fungal community differs between a coexisting native shrub and introduced annual grass. Mycorrhiza 23: 129-141. doi: 10.1007/s00572-012-0455-x.

Carter KA, Smith JF, White MM, Serpe MD (2014) Assessing the diversity of arbuscular mycorrhizal fungi in semiarid shrublands dominated by Artemisia tridentata ssp. wyomingensis. Mycorrhiza: 301-314.

Cavagnaro TR, Smith FA, Ayling SM, Smith SE (2003) Growth and phosphorus nutrition of a Paris-type arbuscular mycorrhizal symbiosis. New Phytologist 157: 127-134. doi: 10.1046/j.1469-8137.2003.00654.x.

Cavender-Bares J (2004) From leaves to ecosystems: Using chlorophyll fluorescence to assess photosynthesis and plant function in ecological studies. Advances in Photosynthesis and Respiration 19: 737-755.

Cesaro P, van Tuinen D, Copetta A, Chatagnier O, Berta G, Gianinazzi S, Lingua G (2008) Preferential colonization of *Solanum tuberosum* L. roots by the fungus *Glomus intraradices* in arable soil of a potato farming area. Appl Environ Microbiol 74: 5776-5783

Charley J, West N (1977) Micro-patterns of nitrogen mineralization activity in soils of some shrub-dominated semi-desert ecosystems of Utah. Soil Biology and Biochemistry 9: 357-365.

Citernesi AS, Vitagliano C, Giovannetti M (1998) Plant growth and root system morphology of Olea europaea L. Rooted cuttings as influenced by arbuscular mycorrhizas. Journal of Horticultural Science & Biotechnology 73: 647-654.

Corradi N, Bonfante P (2012) The Arbuscular Mycorrhizal Symbiosis: Origin and Evolution of a Beneficial Plant Infection. Plos Pathogens 8. doi: 10.1371/journal.ppat.1002600.

Cox RD, Anderson VJ (2004) Increasing native diversity of cheatgrass-dominated rangeland through assisted succession. Rangeland Ecology & Management 57: 203-210.

D'Antonio CM, Vitousek PM (1992) Biological invasions by exotic grasses, the grass/fire cycle, and global change. Annual review of ecology and systematics 23: 63-87.

Dalzell CR (2004) Post-fire establishment of vegetation communities following reseeding on southern Idaho's Snake River Plain.

Dangi SR, Stahl PD, Pendall E (2010) Recovery of soil microbial community structure after fire in a sagebrush-grassland ecosystem. Land Degrad Dev Land Degradation & Development: n/a.

Davies KW, Bates JD, Miller RF (2007) The influence of Artemsia tridentata ssp wyomingensis on microsite and herbaceous vegetation heterogeneity. Journal of Arid Environments 69: 441-457. doi: 10.1016/j.jaridenv.2006.10.017.

de Jonge LW, Jacobsen OH, Moldrup P (1999) Soil water repellency: Effects of water content, temperature, and particle size. Soil Science Society of America Journal 63: 437-442.

DeByle NV (1973) Broadcast burning of logging residues and the water repellency of soils. Northwest Science 47: 77-87.

Dixon RK, Rao MV, Garg VK (1994) Water relations and gas exchange of mycorrhizal Leucaena leucocephala seedlings. Journal of Tropical Forest Science 6: 542-552.

Dobrowolski JP, Caldwell MM, Richards JH (1990) Basin hydrology and plant root systems. Plant Biology of the Basin and Range. Springer.

Drew EA, Murray RS, Smith SE, Jakobsen I (2003) Beyond the rhizosphere: growth and function of arbuscular mycorrhizal external hyphae in sands of varying pore sizes. Plant and Soil 251: 105-114. doi: 10.1023/a:1022932414788.

Drigo B, Pijl AS, Duyts H, Kielak AM, Gamper HA, Houtekamer MJ, Boschker HTS, Bodelier PLE, Whiteley AS, Veen JAv, Kowalchuk GA (2010) Shifting carbon flow from roots into associated microbial communities in response to elevated atmospheric CO2. Proceedings of the National Academy of Sciences 107: 10938-10942. doi: 10.1073/pnas.0912421107.

Driver JD, Holben WE, Rillig MC (2005) Characterization of glomalin as a hyphal wall component of arbuscular mycorrhizal fungi. Soil Biol Biochem 37 SRC - GoogleScholar: 101-106.

Duke S, Jackson R, Caldwell M (1994) Local reduction of mycorrhizal arbuscule frequency in enriched soil microsites. Canadian Journal of Botany 72: 998-1001.

Dumbrell AJ, Ashton PD, Aziz N, Feng G, Nelson M, Dytham C, Fitter AH, Helgason T (2011) Distinct seasonal assemblages of arbuscular mycorrhizal fungi revealed by massively parallel pyrosequencing. New Phytologist 190: 794-804.

Eiswerth ME, Scott Shonkwiler J (2006) Examining post-wildfire reseeding on arid rangeland: a multivariate tobit modelling approach. Ecological modelling 192: 286-298.

El-Tohamy W, Schnitzler WH, El-Behairy U, El-Beltagy MS (1999) Effect of VA mycorrhiza on improving drought and chilling tolerance of bean plants (Phaseolus vulgaris L.). Journal of Applied Botany-Angewandte Botanik 73: 178-183.

Evans DG, Miller MH (1990) The role of the extermal mycelial network in the effect of soil disturbance upon vesicular arbuscular mycorrhizal colonization of maize. New Phytologist 114: 65-71. doi: 10.1111/j.1469-8137.1990.tb00374.x.

Evans RA, Young JA (1978) Effectiveness of rehabilitation practices following wildfire in a degraded big sagebrush-downy brome community. Journal of Range Management: 185-188.

Facelli E, Smith SE, Facelli JM, Christophersen HM, Smith FA (2010) Underground friends or enemies: model plants help to unravel direct and indirect effects of arbuscular mycorrhizal fungi on plant competition. New Phytologist 185: 1050-1061. doi: 10.1111/j.1469-8137.2009.03162.x.

Fan L, Dalpe Y, Fang C, Dube C, Khanizadeh S (2011) Influence of arbuscular mycorrhizae on biomass and root morphology of selected strawberry cultivars under salt stress. Botany-Botanique 89: 397-403. doi: 10.1139/b11-028.

Farris JS, Kallersjo M, Kluge AG, Bult C (1994) Testing significance of incongruence. Cladistics-the International Journal of the Willi Hennig Society 10: 315-319. doi: 10.1006/clad.1994.1021.

Feddermann N, Finlay R, Boller T, Elfstrand M (2010) Functional diversity in arbuscular mycorrhiza - the role of gene expression, phosphorous nutrition and symbiotic efficiency. Fungal Ecology 3: 1-8. doi: 10.1016/j.funeco.2009.07.003.

Felsenstein J (1985) Confidence-limits of phylogenies - an approach using bootstrap. Evolution 39: 783-791. doi: 10.2307/2408678.

Finlay RD (2008) Ecological aspects of mycorrhizal symbiosis: with special emphasis on the functional diversity of interactions involving the extraradical mycelium. Journal of Experimental Botany 59: 1115-1126. doi: 10.1093/jxb/ern059.

Flexas J, Briantais JM, Cerovic Z, Medrano H, Moya I (2000) Steady-state and maximum chlorophyll fluorescence responses to water stress in grapevine leaves: A new remote sensing system. Remote Sensing of Environment 73: 283-297. doi: 10.1016/s0034-4257(00)00104-8.

Flexas J, Escalona JM, Evain S, Gulías J, Moya I, Osmond CB, Medrano H (2002) Steady-state chlorophyll fluorescence (Fs) measurements as a tool to follow variations of net CO2 assimilation and stomatal conductance during water-stress in C3 plants. Physiologia Plantarum 114: 231-240.

Friese CF, Allen MF (1991) The Spread Of Va Mycorrhizal Fungal Hyphae In The Soil - Inoculum Types And External Hyphal Architecture. Mycologia 83: 409-418. doi: 10.2307/3760351.

Frost SM, Stahl PD, Williams SE (2001) Long-term reestablishment of arbuscular mycorrhizal fungi in a drastically disturbed semiarid surface mine soil. Arid Land Research and Management 15: 3-12. doi: 10.1080/15324980119429.

Gelbard JL, Belnap J (2003) Roads as conduits for exotic plant invasions in a semiarid landscape. Conservation Biology 17: 420-432.

Giri B, Kapoor R, Agarwal L, Mukerji KG (2004) Preinoculation with arbuscular mycorrhizae helps Acacia auriculiformis grow in degraded Indian wasteland soil. Communications in Soil Science and Plant Analysis 35: 193-204. doi: 10.1081/css-120027643.

Gollotte A, van Tuinen D, Atkinson D (2004) Diversity of arbuscular mycorrhizal fungi colonising roots of the grass species *Agrostis capillaris* and *Lolium perenne* in a field experiment. Mycorrhiza 14: 111-117. doi: 10.1007/s00572-003-0244-7.

Grace EJ, Smith FA, Smith SE (2009) Deciphering the Arbuscular Mycorrhizal Pathway of P Uptake in Non-responsive Plant Species.

Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology 52: 696-704. doi: 10.1080/10635150390235520.

Gurr JE (1993) The effect of fire on vesicular arbuscular mycorrhizae, soil nutrients, and the re-establishment of Wyoming big sagebrush. Boise State University Graduate Thesis.

Hardie K, Leyton L (1981) The influence of vesicular-arbuscular mycorrhiza on growth and water relations of red clover. New Phytologist 89: 599-608.

Helgason T, Fitter AH (2009) Natural selection and the evolutionary ecology of the arbuscular mycorrhizal fungi (Phylum Glomeromycota). Journal of Experimental Botany 60: 2465-2480. doi: 10.1093/jxb/erp144.

Hetrick BAD (1991) Mycorrhizas and root architecture. Experientia 47: 355-362. doi: 10.1007/bf01972077.

Hetrick BAD, Wilson GWT, Todd TC (1996) Mycorrhizal response in wheat cultivars: Relationship to phosphorus. Canadian Journal of Botany-Revue Canadienne De Botanique 74: 19-25.

Ho MD, McCannon BC, Lynch JP (2004) Optimization modeling of plant root architecture for water and phosphorus acquisition. Journal of Theoretical Biology 226: 331-340. doi: 10.1016/j.jtbi.2003.09.011.

Hoeksema JD, Chaudhary VB, Gehring CA, Johnson NC, Karst J, Koide RT, Pringle A, Zabinski C, Bever JD, Moore JC, Wilson GWT, Klironomos JN, Umbanhowar J (2010) A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. Ecology Letters 13: 394-407. doi: 10.1111/j.1461-0248.2009.01430.x.

Hou JQ, Romo JT (1998) Cold-hardiness of silver sagebrush seedlings. Journal of Range Management 51: 704-708. doi: 10.2307/4003616.

Husband R, Herre EA, Young JPW (2002) Temporal variation in the arbuscular mycorrhizal communities colonising seedlings in a tropical forest. FEMS Microbiology Ecology 42: 131-136.

Jakobsen T, Erik Nielsen N (1983) Vesicular-arbuscular mycorrhiza in field-grown crops. New Phytologist 93: 401-413.

Jansa J, Smith FA, Smith SE (2008) Are there benefits of simultaneous root colonization by different arbuscular mycorrhizal fungi? New Phytologist 177: 779-789.

Jayne B, Quigley M (2014) Influence of arbuscular mycorrhiza on growth and reproductive response of plants under water deficit: a meta-analysis. Mycorrhiza 24: 109-119. doi: 10.1007/s00572-013-0515-x.

Ji B, Bentivenga SP, Casper BB (2010) Evidence for ecological matching of whole AM fungal communities to the local plant-soil environment. Ecology 91: 3037-3046. doi: 10.1890/09-1451.1.

Johnson NC (2010) Resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales. New Phytologist 185: 631-647. doi: 10.1111/j.1469-8137.2009.03110.x.

Johnson NC, Graham JH (2013) The continuum concept remains a useful framework for studying mycorrhizal functioning. Plant and Soil 363: 411-419. doi: 10.1007/s11104-012-1406-1.

Johnson NC, Rowland DL, Corkidi L, Allen EB (2008) Plant winners and losers during grassland N-eutrophication differ in biomass allocation and mycorrhizas. Ecology 89: 2868-2878.

Johnson TB, Augé RM, Green CD, Stodola AJW, Olinick JB, Saxton AM (2001) Correlations of stomatal conductance with hydraulic, chemical and environmental variables in five urban tree species. Scientia Horticulturae 90: 305-320. doi: 10.1016/s0304-4238(00)00265-x.

Jones MD, Smith SE (2004) Exploring functional definitions of mycorrhizas: Are mycorrhizas always mutualisms? Canadian Journal of Botany-Revue Canadienne De Botanique 82: 1089-1109. doi: 10.1139/b04-110.

Kabir Z, O'halloran I, Fyles J, Hamel C (1997) Seasonal changes of arbuscular mycorrhizal fungi as affected by tillage practices and fertilization: hyphal density and mycorrhizal root colonization. Plant and Soil 192: 285-293.

Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR, Kowalchuk GA, Hart MM, Bago A, Palmer TM, West SA, Vandenkoornhuyse P, Jansa J, Buecking H (2011) Reciprocal Rewards Stabilize Cooperation in the Mycorrhizal Symbiosis. Science 333: 880-882. doi: 10.1126/science.1208473.

Klironomos JN (2002) Feedback with soil biota contributes to plant rarity and invasiveness in communities. Nature 417: 67-70.

Klironomos JN, Moutoglis P, Kendrick B, Widden P (1993) A comparison of spatial heterogeneity of vesicular-arbuscular mycorrhizal fungi in two maple-forest soils. Canadian Journal of Botany 71: 1472-1480.

Klironomos JN, Ursic M, Rillig M, Allen MF (1998) Interspecific differences in the response of arbuscular mycorrhizal fungi to Artemisia tridentata grown under elevated atmospheric CO2. New Phytologist 138: 599-605.

Knick ST, Dobkin DS, Rotenberry JT, Schroeder MA, Vander Haegen WM, van Riper III C (2003) Teetering on the edge or too late? Conservation and research issues for avifauna of sagebrush habitats. The Condor 105: 611-634.

Korhonen A, Lehto T, Repo T (2013) Frost hardiness of mycorrhizal (Hebeloma sp.) and non-mycorrhizal Scots pine roots. Mycorrhiza 23: 551-559. doi: 10.1007/s00572-013-0497-8.

Kozlowski TT, Pallardy SG (2002) Acclimation and adaptive responses of woody plants to environmental stresses. Botanical Review 68: 270-334. doi: 10.1663/0006-8101(2002)068[0270:aaarow]2.0.co;2.

Krüger M, Krüger C, Walker C, Stockinger H, Schüssler A (2012) Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. The New phytologist 193: 970-984.

Lambrecht SC, Shattuck AK, Loik ME (2007) Combined drought and episodic freezing effects on seedlings of low- and high-elevation subspecies of sagebrush (*Artemisia tridentata*). Physiologia Plantarum 130: 207-217. doi: 10.1111/j.1399-3054.2007.00904.x.

Larrucea ES, Brussard PF (2008) Habitat selection and current distribution of the pygmy rabbit in Nevada and California, USA. Journal of Mammalogy 89: 691-699. doi: 10.1644/07-mamm-a-199r.1.

Latef AAHA, He C (2011) Arbuscular mycorrhizal influence on growth, photosynthetic pigments, osmotic adjustment and oxidative stress in tomato plants subjected to low temperature stress. Acta Physiologiae Plantarum 33: 1217-1225. doi: 10.1007/s11738-010-0650-3.

Lekberg Y, Koide RT (2005) Is plant performance limited by abundance of arbuscular mycorrhizal fungi? A meta-analysis of studies published between 1988 and 2003. New Phytologist 168: 189-204. doi: 10.1111/j.1469-8137.2005.01490.x.

Lekberg Y, Koide RT, Rohr JR, Aldrich-Wolfe L, Morton JB (2007) Role of niche restrictions and dispersal in the composition of arbuscular mycorrhizal fungal communities. Journal of Ecology 95: 95-105.

Leu M, Hanser SE, Knick ST (2008) The human footprint in the west: a large-scale analysis of anthropogenic impacts. Ecological Applications 18: 1119-1139.

Li H, Smith FA, Dickson S, Holloway RE, Smith SE (2008) Plant growth depressions in arbuscular mycorrhizal symbioses: not just caused by carbon drain? New Phytologist 178: 852-862. doi: 10.1111/j.1469-8137.2008.02410.x.

Li L-F, Li T, Zhang Y, Zhao Z-W (2010a) Molecular diversity of arbuscular mycorrhizal fungi and their distribution patterns related to host-plants and habitats in a hot and arid ecosystem, southwest China. Fems Microbiology Ecology 71: 418-427. doi: 10.1111/j.1574-6941.2009.00815.x.

Li T, Lin G, Zhang X, Chen Y, Zhang S, Chen B (2014) Relative importance of an arbuscular mycorrhizal fungus (Rhizophagus intraradices) and root hairs in plant drought tolerance. Mycorrhiza 24: 595-602. doi: 10.1007/s00572-014-0578-3.

Li Y, He X, Zhao L (2010b) Tempo-spatial dynamics of arbuscular mycorrhizal fungi under clonal plant Psammochloa villosa Trin. Bor in Mu Us sandland. European Journal of Soil Biology 46: 295-301. doi: 10.1016/j.ejsobi.2010.05.004.

Liesack W, Weyland H, Stackebrandt E (1991) Potential risks of gene amplification by PCR as determined by 16S rDNA analysis of a mixed-culture of strict barophilic bacteria. Microbial Ecology 21: 191-198.

Lim C-C, Krebs SL, Arora R (2014) Cold hardiness increases with age in juvenile Rhododendron populations. Frontiers in Plant Science 5. doi: 10.3389/fpls.2014.00542.

Lingfei L, Anna Y, Zhiwei Z (2005) Seasonality of arbuscular mycorrhizal symbiosis and dark septate endophytes in a grassland site in southwest China. FEMS Microbiology Ecology 54: 367-373.

Liu Y, He J, Shi G, An L, Oepik M, Feng H (2011) Diverse communities of arbuscular mycorrhizal fungi inhabit sites with very high altitude in Tibet Plateau. Fems Microbiology Ecology 78: 355-365. doi: 10.1111/j.1574-6941.2011.01163.x.

Loreau M, Naeem S, Inchausti P, Bengtsson J, Grime J, Hector A, Hooper D, Huston M, Raffaelli D, Schmid B (2001) Biodiversity and ecosystem functioning: current knowledge and future challenges. science 294: 804-808.

Macek I, Dumbrell AJ, Nelson M, Fitter AH, Vodnik D, Helgason T (2011) Local Adaptation to Soil Hypoxia Determines the Structure of an Arbuscular Mycorrhizal Fungal Community in Roots from Natural CO2 Springs. Applied and Environmental Microbiology 77: 4770-4777. doi: 10.1128/aem.00139-11.

Maherali H, Klironomos JN (2007) Influence of Phylogeny on fungal community assembly and ecosystem functioning. Science 316: 1746-1748. doi: 10.1126/science.1143082.

Martínez-García LB, de Dios Miranda J, Pugnaire FI (2012) Impacts of changing rainfall patterns on mycorrhizal status of a shrub from arid environments. European Journal of Soil Biology 50: 64-67.

Marulanda A, Azcón R, Ruiz-Lozano JM (2003) Contribution of six arbuscular mycorrhizal fungal isolates to water uptake by Lactuca sativa plants under drought stress. Physiologia Plantarum 119: 526-533. doi: 10.1046/j.1399-3054.2003.00196.x.

McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990) A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytologist 115: 495-501.

Meyer DW, Badaruddin M (2001) Frost tolerance of ten seedling legume species at four growth stages. Crop Science 41: 1838-1842.

Meyer SE (1994) Germination and establishment ecology of big sagebrush: implications for community restoration. Proceedings—ecology and management of annual rangelands Gen Tech Rep INT-313 Ogden, UT: US Department of Agriculture, Forest Service, Intermountain Research Station: 244-251.

Minchin PR (1987) An evaluation of the relative robustness of techniques for ecological ordination. Theory and models in vegetation science. Springer.

Monsen SB, Stevens R, Shaw NL (2004) Restoring western ranges and wildlands. United States Dept. of Agriculture, Forest Service, Rocky Mountain Research Station.

Moorman T, Reeves FB (1979) Role Of Endomycorrhizae In Revegetation Practices In The Semi-Arid West .2. Bioassay To Determine The Effect Of Land Disturbance On Endomycorrhizal Populations. American Journal of Botany 66: 14-18. doi: 10.2307/2442619.

Morton J, Koske R, Stürmer S, Bentivenga S (2004) Mutualistic arbuscular endomycorrhizal fungi. Biodiversity of Fungi: Inventory and Monitoring Methods: 317-336.

Muller K (2004) PRAP-computation of Bremer support for large data sets. Molecular Phylogenetics and Evolution 31: 780-782. doi: 10.1016/j.ympev.2003.12.006.

Mummey DL, Antunes PM, Rillig MC (2009) Arbuscular mycorrhizal fungi pre-inoculant identity determines community composition in roots. Soil Biology and Biochemistry 41: 1173-1179.

Mummey DL, Rillig MC (2006) The invasive plant species Centaurea maculosa alters arbuscular mycorrhizal fungal communities in the field. Plant and Soil 288: 81-90. doi: 10.1007/s11104-006-9091-6.

Mummey DL, Rillig MC (2007) Evaluation of LSU rRNA-gene PCR primers for analysis of arbuscular mycorrhizal fungal communities via terminal restriction fragment length polymorphism analysis. Journal of Microbiological Methods 70: 200-204. doi: 10.1016/j.mimet.2007.04.002.

Muscha J, Hild A (2006) Biological soil crusts in grazed and ungrazed Wyoming sagebrush steppe. Journal of Arid Environments 67: 195-207.

Navarro Garcia A, Banon Arias SdP, Morte A, Jesus Sánchez-Blanco M (2011) Effects of nursery preconditioning through mycorrhizal inoculation and drought in Arbutus unedo L. plants. Mycorrhiza 21: 53-64. doi: 10.1007/s00572-010-0310-x.

NOAA/NCDC (2014) Climatological Rankings. NOAA National Climatic Data Center, http://www.ncdc.noaa.gov/temp-and-precip/climatological-rankings/.

Nordin A, Schmidt IK, Shaver GR (2004) Nitrogen uptake by arctic soil microbes and plants in relation to soil nitrogen supply. Ecology 85: 955-962.

Norman JR, Atkinson D, Hooker JE (1996) Arbuscular mycorrhizal fungal-induced alteration to root architecture in strawberry and induced resistance to the root pathogen Phytophthora fragariae. Plant and Soil 185: 191-198. doi: 10.1007/bf02257524.

Noss RF, LaRoe ET, Scott JM (1995) Endangered ecosystems of the United States: a preliminary assessment of loss and degradation. US Department of the Interior, National Biological Service Washington, DC, USA.

O'Dea ME (2007) Influence of mycotrophy on native and introduced grass regeneration in a semiarid grassland following burning. Restoration Ecology 15: 149-155. doi: 10.1111/j.1526-100X.2006.00199.x.

O'Mullan GD, Ward BB (2005) Relationship of temporal and spatial variabilities of ammonia-oxidizing bacteria to nitrification rates in Monterey Bay, California. Applied and Environmental Microbiology 71: 697-705. doi: 10.1128/aem.71.2.697-705.2005.

Oehl F, Sieverding E, Ineichen K, Ris EA, Boller T, Wiemken A (2005) Community structure of arbuscular mycorrhizal fungi at different soil depths in extensively and intensively managed agroecosystems. New Phytologist 165: 273-283.

Oksanen J, Kindt R, Legendre P, O'Hara B, Stevens MHH, Oksanen MJ, Suggests M (2007) The vegan package. Community ecology package Version: 1.8-5.

Olff H, Ritchie ME (1998) Effects of herbivores on grassland plant diversity. Trends in ecology & evolution 13: 261-265.

Osonubi O, Bakare ON, Mulongoy K (1992) Interactions Between Drought Stress And Vesicular-Arbuscular Mycorrhiza On The Growth Of Faidherbia-Albida (Syn Acacia-Albida) And Acacia-Nilotica In Sterile And Nonsterile Soils. Biology and Fertility of Soils 14: 159-165. doi: 10.1007/bf00346056.

Ouahmane L, Hafidi M, Thioulouse J, Ducousso M, Kisa M, Prin Y, Galiana A, Boumezzough A, Duponnois R (2007) Improvement of Cupressus atlantica Gaussen growth by inoculation with native arbuscular mycorrhizal fungi. Journal of Applied Microbiology 103: 683-690. doi: 10.1111/j.1365-2672.2007.03296.x.

Paradis R, Dalpe Y, Charest C (1995) The combined effect of arbuscular mycorrhizas and short-term cold exposure on wheat. New phytologist 129: 637-642.

Pattinson G, Hammill K, Sutton B, McGee P (1999) Simulated fire reduces the density of arbuscular mycorrhizal fungi at the soil surface. Mycological Research 103: 491-496.

Pederson JC, Welch BL (1982) Effects of monoterpenoid exposure on ability of rumen inocula to digest a set of forages. Journal of Range Management: 500-502.

Pellant M (1990) The cheatgrass-wildfire cycle—are there any solutions. McArthur et al(eds) Proceedings of a Symposium on cheatgrass invasion, shrub die-off, and other aspects of shrub biology and management US For Serv, Int Res Sta, Gen Tech Rep INT-276 Ogden, UT.

Pellant M, Lysne CR, Shaw N, Pellant M (2005) Strategies to enhance plant structure and diversity in crested wheatgrass seedings. Proceedings of the USDA Forest Service RMRS-P-38 Fort Collins, CO: Rocky Mountain Research Station: 64-70.

Perryman BL, Maier AM, Hild AL, Olson RA (2001) Demographic characteristics of 3 Artemisia tridentata Nutt. subspecies. Journal of Range Management 54: 166-170. doi: 10.2307/4003178.

Pinior A, Grunewaldt-Stöcker G, von Alten H, Strasser RJ (2005) Mycorrhizal impact on drought stress tolerance of rose plants probed by chlorophyll a fluorescence, proline content and visual scoring. Mycorrhiza 15: 596-605.

Pivato B, Mazurier S, Lemanceau P, Siblot S, Berta G, Mougel C, van Tuinen D (2007) Medicago species affect the community composition of arbuscular mycorrhizal fungi associated with roots. New Phytologist 176: 197-210. doi: 10.1111/j.1469-8137.2007.02151.x.

Porcel R, Ruiz-Lozano JM (2004) Arbuscular mycorrhizal influence on leaf water potential, solute accumulation, and oxidative stress in soybean plants subjected to drought stress. Journal of Experimental Botany 55: 1743-1750. doi: 10.1093/jxb/erh188.

Posada D (2008) jModelTest: Phylogenetic model averaging. Molecular Biology and Evolution 25: 1253-1256. doi: 10.1093/molbev/msn083.

Prevéy JS, Germino MJ, Huntly NJ, Inouye RS (2010) Exotic plants increase and native plants decrease with loss of foundation species in sagebrush steppe. Plant Ecology 207: 39-51.

Pringle A, Bever JD (2002) Divergent phenologies may facilitate the coexistence of arbuscular mycorrhizal fungi in a North Carolina grassland. American Journal of Botany 89: 1439-1446.

Pringle A, Bever JD, Gardes M, Parrent JL, Rillig MC, Klironomos JN (2009) Mycorrhizal Symbioses and Plant Invasions. Annual Review of Ecology Evolution and Systematics 40: 699-715. doi: 10.1146/annurev.ecolsys.39.110707.173454.

Querejeta J, Egerton-Warburton LM, Allen MF (2003) Direct nocturnal water transfer from oaks to their mycorrhizal symbionts during severe soil drying. Oecologia 134: 55-64.

Querejeta JI, Allen MF, Caravaca F, Roldán A (2006) Differential modulation of host plant δ13C and δ18O by native and nonnative arbuscular mycorrhizal fungi in a semiarid environment. New Phytologist 169: 379-387. doi: 10.1111/j.1469-8137.2005.01599.x.

R Core Team (2012) R: A Language and Environment for Statistical Computing. 2.15.1 edn. R Foundation for Statistical Computing, Vienna, Austria.

Rakshit A, Bhadoria P (2008) Indigenous arbuscular mycorrhiza is more important for early growth period of groundnut (Arachis hypogea L.) for P influx in an Oxisol. Acta agriculturae Slovenica 91: 397-406.

Rambaut A, Drummond A (2005) Tracer. A program for analysing results from Bayesian MCMC programs such as BEAST & MrBayes. Version 1.3. Oxford University,[online] http://evolve. zoo. ox. ac. uk/software. html.

Redecker D, Kodner R, Graham LE (2000) Glomalean fungi from the Ordovician. Science 289: 1920-1921. doi: 10.1126/science.289.5486.1920.

Redecker D, Schuessler A, Stockinger H, Stuermer SL, Morton JB, Walker C (2013) An evidence-based consensus for the classification of arbuscular mycorrhizal fungi (Glomeromycota). Mycorrhiza 23: 515-531. doi: 10.1007/s00572-013-0486-y.

Requena N, Perez-Solis E, Azcón-Aguilar C, Jeffries P, Barea JM (2001) Management of indigenous plant-microbe symbioses aids restoration of desertified ecosystems. Applied and Environmental Microbiology 67: 495-498. doi: 10.1128/aem.67.2.495-498.2001.

Richards JH, Caldwell MM (1987) Hydraulic lift: substantial nocturnal water transport between soil layers by Artemisia tridentata roots. Oecologia 73: 486-489.

Rillig MC, Field CB (2003) Arbuscular mycorrhizae respond to plants exposed to elevated atmospheric CO2 as a function of soil depth. Plant and Soil 254: 383-391.

Robichaud P, Hungerford R (2000) Water repellency by laboratory burning of four northern Rocky Mountain forest soils. Journal of Hydrology 231: 207-219.

Rosendahl S, Stukenbrock EH (2004) Community structure of arbuscular mycorrhizal fungi in undisturbed vegetation revealed by analyses of LSU rDNA sequences. Molecular Ecology 13: 3179-3186.

Rosentreter R, Kelsey RG (1991) Xeric big sagebrush, a new subspecies in the Artemisia tridentata complex. Journal of Range Management: 330-335.

Rout ME, Callaway RM (2012) Interactions between exotic invasive plants and soil microbes in the rhizosphere suggest that 'everything is not everywhere'. Annals of botany: mcs061.

Ruiz-Lozano JM, Azcón R (1995) Hyphal contribution to water uptake in mycorrhizal plants as affected by the fungal species and water status. Physiologia Plantarum 95: 472-478. doi: 10.1111/j.1399-3054.1995.tb00865.x.

Ryan M, Small D, Ash J (2000) Phosphorus controls the level of colonisation by arbuscular mycorrhizal fungi in conventional and biodynamic irrigated dairy pastures. Animal Production Science 40: 663-670.

Ryan R (2012) Seasonal variation in arbuscular mycorrhizal fungi root colonization of cheatgrass (Bromus tectorum), an invasive winter annual. Journal of Ecosystem & Ecography.

Ryel RJ, Caldwell MM (1998) Nutrient Acquisition From Soils With Patchy Nutrient Distributions As Assessed With Simulation Models. Ecology 79: 2735-2744. doi: 10.1890/0012-9658(1998)079[2735:NAFSWP]2.0.CO;2.

Salih MS, Taha FK, Payne GF (1973) Water repellency of soils under burned sagebrush. Journal of Range Management 26: 330-331.

Sánchez-Blanco MJ, Ferrandez T, Morales MA, Morte A, Alarcon JJ (2004) Variations in water status, gas exchange, and growth in Rosmarinus officinalis plants infected with Glomus deserticola under drought conditions. Journal of Plant Physiology 161: 675-682. doi: 10.1078/0176-1617-01191.

Sánchez-castro I, Ferrol N, Cornejo P, Barea J-m (2012) Temporal dynamics of arbuscular mycorrhizal fungi colonizing roots of representative shrub species in a

semi-arid Mediterranean ecosystem. Mycorrhiza 22: 449-460. doi: 10.5248/116.75 http://dx.doi.org/10.1007/s00572-011-0421-z.

Sanders IR (2003) Preference, specificity and cheating in the arbuscular mycorrhizal symbiosis. Trends in Plant Science 8: 143-145. doi: 10.1016/s1360-1385(03)00012-8.

Sanders IR, Clapp JP, Wiemken A (1996) The genetic diversity of arbuscular mycorrhizal fungi in natural ecosystems–a key to understanding the ecology and functioning of the mycorrhizal symbiosis. New Phytologist 133: 123-134.

Santos-González JC, Finlay RD, Tehler A (2007) Seasonal dynamics of arbuscular mycorrhizal fungal communities in roots in a seminatural grassland. Applied and Environmental Microbiology 73: 5613-5623.

Sardans J, Peñuelas J, Estiarte M (2007) Seasonal patterns of root-surface phosphatase activities in a Mediterranean shrubland. Responses to experimental warming and drought. Biology and Fertility of Soils 43: 779-786.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF (2009) Introducing mothur: open-oource, platform-independent, community-cupported coftware for describing and comparing microbial communities. Applied and Environmental Microbiology 75: 7537-7541. doi: 10.1128/aem.01541-09.

Schnoor TK, Lekberg Y, Rosendahl S, Olsson PA (2011) Mechanical soil disturbance as a determinant of arbuscular mycorrhizal fungal communities in semi-natural grassland. Mycorrhiza 21: 211-220. doi: 10.1007/s00572-010-0325-3.

Schüßler A, Schwarzott D, Walker C (2001) A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycological research 105: 1413-1421.

Schwartz MW, Hoeksema JD, Gehring CA, Johnson NC, Klironomos JN, Abbott LK, Pringle A (2006) The promise and the potential consequences of the global transport of mycorrhizal fungal inoculum. Ecology Letters 9: 501-515. doi: 10.1111/j.1461-0248.2006.00910.x.

Selosse MA, Baudoin E, Vandenkoornhuyse P (2004) Symbiotic microorganisms, a key for ecological success and protection of plants. Comptes Rendus Biologies 327: 639-648. doi: 10.1016/j.crvi.2003.12.008.

Sheley RLJJ (2010) Resistance of Native Plant Functional Groups to Invasion by Medusahead (Taeniatherum caput-medusae). ipsm Invasive Plant Science and Management 3: 294-300.

Shultz LM (2009) Monograph of Artemisia subgenus Tridentatae (Asteraceae-Anthemideae). Systematic Botany Monographs 89.

SigmaPlot. 12.5 edn. Systat Software, Inc., San Jose, CA, USA.

Smith S, Read D (1997) Mycorrhizal symbiosis. Academic Press, London 804 p.

Smith SE, Jakobsen I, Grønlund M, Smith FA (2011) Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. Plant physiology 156: 1050-1057.

Smith SE, Smith FA (2011) Roles of Arbuscular Mycorrhizas in Plant Nutrition and Growth: New Paradigms from Cellular to Ecosystem Scales. Annual Review of Plant Biology, Vol 62 62: 227-250. doi: 10.1146/annurev-arplant-042110-103846.

Smith SE, Smith FA, Jakobsen I (2003) Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. Plant Physiology 133: 16-20. doi: 10.1104/pp.103.024380.

Smith T (1978) A note on the effect of soil tillage on the frequency and vertical distribution of spores of vesicular-arbuscular endophytes. Soil Research 16: 359-361.

Snellgrove RC, Splittstoesser WE, Stribley DP, Tinker PB (1982) The Distribution Of Carbon And The Demand Of The Fungal Symbiont In Leek Plants With Vesicular Arbuscular Mycorrhizas. New Phytologist 92: 75-87. doi: 10.1111/j.1469-8137.1982.tb03364.x.

Stahl PD, Schuman GE, Frost SM, Williams SE (1998) Arbuscular mycorrhizae and water stress tolerance of Wyoming big sagebrush seedlings. Soil Science Society of America Journal 62: 1309-1313.

Stahl PD, Williams S, Christensen M (1988) Efficacy of native vesicular-arbuscular mycorrhizal fungi after severe soil disturbance. New Phytologist 110: 347-354.

Stockinger H, Krueger M, Schuessler A (2010) DNA barcoding of arbuscular mycorrhizal fungi. New Phytol 187: 461-474

Stutz JC, Copeman R, Martin CA, Morton JB (2000) Patterns of species composition and distribution of arbuscular mycorrhizal fungi in arid regions of southwestern North America and Namibia, Africa. Canadian Journal of Botany 78: 237-245.

Sun X-G, Tang M (2013) Effect of arbuscular mycorrhizal fungi inoculation on root traits and root volatile organic compound emissions of Sorghum bicolor. South African Journal of Botany 88: 373-379. doi: 10.1016/j.sajb.2013.09.007.

Suriyagoda LDB, Ryan MH, Renton M, Lambers H (2014) Plant Responses to Limited Moisture and Phosphorus Availability: A Meta-Analysis. Advances in Agronomy, Vol 124 124: 143-200. doi: 10.1016/b978-0-12-800138-7.00004-8.

Swofford D (2002) PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.

Sykorova Z, Ineichen K, Wiemken A, Redecker D (2007) The cultivation bias: different communities of arbuscular mycorrhizal fungi detected in roots from the field, from bait plants transplanted to the field, and from a greenhouse trap experiment. Mycorrhiza 18: 1-14. doi: 10.1007/s00572-007-0147-0.

Taylor TN, Remy W, Hass H, Kerp H (1995) Fossil Arbuscular Mycorrhizae From The Early Devonian. Mycologia 87: 560-573. doi: 10.2307/3760776.

Tinker PB, Nye PH (2000) Solute movement in the rhizosphere. Topics in Sustainable Agronomy. Oxford, UK: Oxford University Press.

Tisserant B, Schellenbaum L, Gianinazzi-Pearson V, Gianinazzi S, Berta G (1990) Influence of infection by an endomycorrhizal fungus on root development and architecture in Platanus acerifolia. Allionia (Turin) 30: 171-181.

Torpy FR, Morrison DA, Bloomfield BJ (1999) The influence of fire frequency an arbuscular mycorrhizal colonization in the shrub Dillwynia retorta (Wendland) Druce (Fabaceae). Mycorrhiza 8: 289-296. doi: 10.1007/s005720050249.

Torrecillas E, Alguacil M, Roldán A (2012a) Host preferences of arbuscular mycorrhizal fungi colonizing annual herbaceous plant species in semiarid Mediterranean prairies. Applied and environmental microbiology 78: 6180-6186.

Torrecillas E, del Mar Alguacil M, Roldan A (2012b) Differences in the AMF diversity in soil and roots between two annual and perennial gramineous plants co-occurring in a Mediterranean, semiarid degraded area. Plant and Soil 354: 97-106. doi: 10.1007/s11104-011-1047-9.

Torrecillas E, Torres P, Alguacil M, Querejeta J, Roldán A (2013) Influence of Habitat and Climate Variables on Arbuscular Mycorrhizal Fungus Community Distribution, as Revealed by a Case Study of Facultative Plant Epiphytism under Semiarid Conditions. Applied and environmental microbiology 79: 7203-7209.

Trent JD, Svejcar TJ, Blank RR (1994) Mycorrhizal colonization, hyphal lengths, and soil moisture associated with two Artemisia tridentata subspecies. Western North American Naturalist 54: 291-300.

Van Der Heijden MG, Streitwolf-Engel R, Riedl R, Siegrist S, Neudecker A, Ineichen K, Boller T, Wiemken A, Sanders IR (2006) The mycorrhizal contribution to plant productivity, plant nutrition and soil structure in experimental grassland. New Phytologist 172: 739-752.

Van der Putten W, Van Dijk C, Peters B (1993) Plant-specific soil-borne diseases contribute to succession in foredune vegetation.

Van der Putten WH, Vet LE, Harvey JA, Wäckers FL (2001) Linking above-and belowground multitrophic interactions of plants, herbivores, pathogens, and their antagonists. Trends in Ecology & Evolution 16: 547-554.

Van Tuinen D, Jacquot E, Zhao B, Gollotte A, Gianinazzi-Pearson V (1998) Characterization of root colonization profiles by a microcosm community of arbuscular mycorrhizal fungi using 25S rDNA-targeted nested PCR. Molecular Ecology 7: 879-887. doi: 10.1046/j.1365-294x.1998.00410.x.

Vandenkoornhuyse P, Husband R, Daniell TJ, Watson IJ, Duck JM, Fitter AH, Young JPW (2002) Arbuscular mycorrhizal community composition associated with two plant species in a grassland ecosystem. Molecular Ecology 11: 1555-1564. doi: 10.1046/j.1365-294X.2002.01538.x.

Verbruggen E, Röling WF, Gamper HA, Kowalchuk GA, Verhoef HA, van der Heijden MG (2010) Positive effects of organic farming on below-ground mutualists: large-scale comparison of mycorrhizal fungal communities in agricultural soils. New Phytologist 186: 968-979.

Vilarino A, Arines J (1991) Numbers and viability of vesicular-arbuscular fungal propagules in field soil samples after wildfire. Soil Biology and Biochemistry 23: 1083-1087.

Vivas A, Voros A, Biro B, Barea JM, Ruiz-Lozano JM, Azcón R (2003) Beneficial effects of indigenous Cd-tolerant and Cd-sensitive Glomus mosseae associated with a Cd-adapted strain of Brevibacillus sp in improving plant tolerance to Cd contamination. Applied Soil Ecology 24: 177-186. doi: 10.1016/s0929-1393(03)00088-x.

Walker BH, Ludwig D, Holling CS, Peterman RM (1981) Stability of semi-arid savanna grazing systems. The Journal of Ecology: 473-498.

Wang GM, Coleman DC, Freckman DW, Dyer MI, McNaughton SJ, Acra MA, Goeschl JD (1989) Carbon Partitioning Patterns Of Mycorrhizal Versus Non-Mycorrhizal Plants - Real-Time Dynamic Measurements Using (CO2)-C-11. New Phytologist 112: 489-493. doi: 10.1111/j.1469-8137.1989.tb00342.x.

Wang Y, Huang Y, Qiu Q, Xin G, Yang Z, Shi S (2011) Flooding Greatly Affects the Diversity of Arbuscular Mycorrhizal Fungi Communities in the Roots of Wetland Plants. Plos One 6. doi: 10.1371/journal.pone.0024512.

Wardle DA (2002) Communities and ecosystems: linking the aboveground and belowground components. Princeton University Press.

Weinbaum BS, Allen MF, Allen EB (1996) Survival of arbuscular mycorrhizal fungi following reciprocal transplanting across the Great Basin, USA. Ecological Applications 6: 1365-1372. doi: 10.2307/2269614.

Welc M, Ravnskov S, Kieliszewska-Rokicka B, Larsen J (2010) Suppression of other soil microorganisms by mycelium of arbuscular mycorrhizal fungi in root-free soil. Soil Biology & Biochemistry 42: 1534-1540. doi: 10.1016/j.soilbio.2010.05.024.

Whisenant SG (1990) Changing fire frequencies on Idaho's Snake River Plains: ecological and management implications. McArthur, E Durant; Romney, Evan M; Smith, Stanley D: 5-7.

Wicklow-Howard M (1989) The occurrence of vesicular-arbuscular mycorrhizae in burned areas of the Snake River Birds of Prey Area, Idaho. Mycotaxon 34: 253-257.

Wright DP, Read DJ, Scholes JD (1998) Mycorrhizal sink strength influences whole plant carbon balance of Trifolium repens L. Plant Cell and Environment 21: 881-891.

Wright SF, Upadhyaya A (1998) A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. Plant and Soil 198: 97-107. doi: 10.1023/a:1004347701584.

Wu Q-S, Srivastava AK, Zou Y-N (2013) AMF-induced tolerance to drought stress in citrus: A review. Scientia Horticulturae 164: 77-87. doi: 10.1016/j.scienta.2013.09.010.

Yang FY, Li GZ, Zhang DE, Christie P, Li XL, Gai JP (2010) Geographical and plant genotype effects on the formation of arbuscular mycorrhiza in Avena sativa and Avena nuda at different soil depths. Biology and Fertility of Soils 46: 435-443. doi: 10.1007/s00374-010-0450-3.

Zajicek J, Hetrick BD, Owensby C (1986) The influence of soil depth on mycorrhizal colonization of forbs in the tallgrass prairie. Mycologia: 316-320.

Zhou Z, Ma H, Liang K, Huang G, Pinyopusarerk K (2012) Improved Tolerance of Teak (Tectona grandis L.f.) Seedlings to Low-Temperature Stress by the Combined Effect of Arbuscular Mycorrhiza and Paclobutrazol. Journal of Plant Growth Regulation 31: 427-435. doi: 10.1007/s00344-011-9252-6

Zouhar K (2003) Bromus tectorum. Fire Effects Information System Fort Collins, CO: USDA Forest Service, Rocky Mountain Research Station Fire Sciences Laboratory http://www fs fed us/database/feis/ Author: Michele A James Scanned Images: Dave Egan Reviewers: Dave Brewer, Dave Egan, Chris McClone, and Judy Springer Series Editor: Dave Egan.

APPENDIX A

Tables

Table 1. Total biomass and shoot-to-root ratio of inoculated and non-inoculated sagebrush seedlings transplanted on May 6, 2011. Mean (± 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 ± 0.65 [a]	1.58 ± 0.12 [a]
Inoculated + Water			8.56 ± 1.53 [a]	1.72 ± 0.58 [a]
Non-Inoculated	1.04 ± 0.15 [a]	1.90 ± 0.30 [a]	2.06 ± 0.66 [b]	1.58 ± 0.15 [a]
Inoculated	1.23 ± 0.30 [a]	2.01 ± 0.28 [a]	1.45 ± 0.71 [b]	1.96 ± 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	*p* value	DF	F value	*p* value	DF	F value	*p* 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
NPQ	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 ($\delta^{13}C$) of non-inoculated and inoculated sagebrush seedlings transplanted on October 1, 2011. Mean (± 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:Root	Leaf $\delta^{13}C$ (‰)
Non-inoculated	3.29 ± 0.481 [a]	3.16 ± 0.276 [a]	-28.644 (± 0.35) [a]
Inoculated	3.54 ± 0.605 [a]	3.12 ± 0.379 [a]	-28.920 (± 0.51) [a]

Table 4. Percent total mycorrhizal and arbuscular colonization at the time of field transplanting in spring and fall 2012. Mean (±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 (± 0.3)^{c}	0^{c}	0.8 (± 0.6)^{c}	0^{b}
Natural (inoculated with soil from the Big Foot site)	15.2 (± 3.3)^{b}	2.0 (± 1.2)^{b}	12.8 (± 2.2)^{b}	2.3 (± 1.1)^{ab}
Inoculated (inoculated with soil from the pot cultures)	24.2 (± 3.5)^{a}	8.3 (± 3.6)^{a}	34.5 (± 8.5)^{a}	6.1 (± 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 ± 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

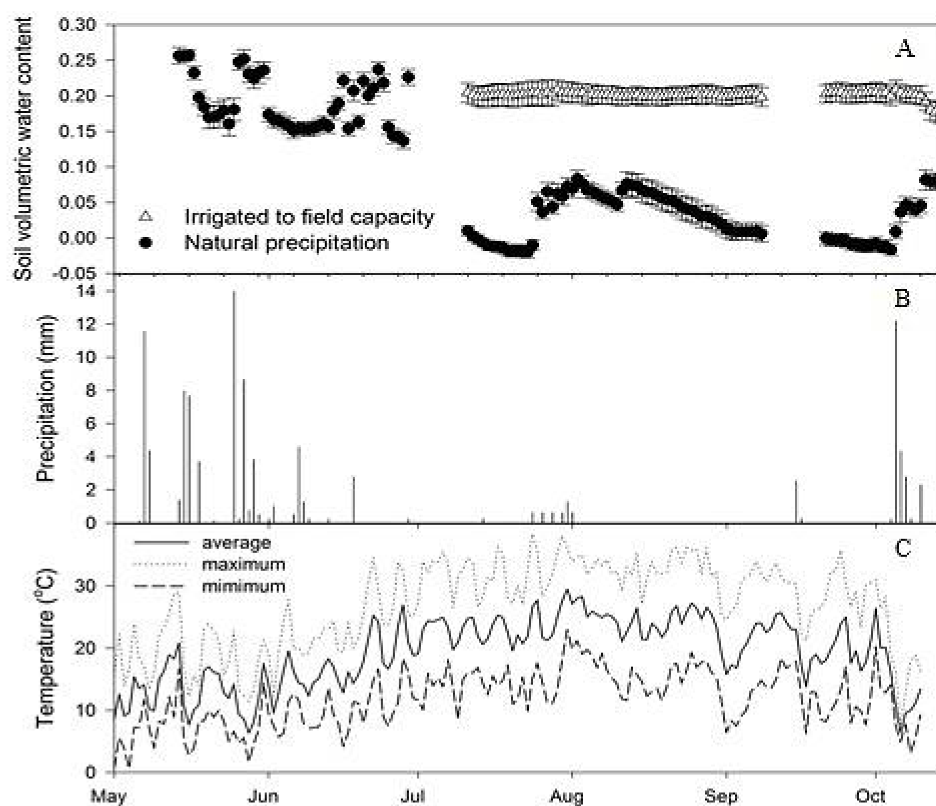

Figure 1. **Environmental conditions that *Artemisia tridentata* seedlings experienced from May 6 to October 15, 2011. A, Soil volumetric water content (volume of water to volume of soil). B, Daily precipitation (mm). C, Daily average, minimum, and maximum temperatures (ºC).**

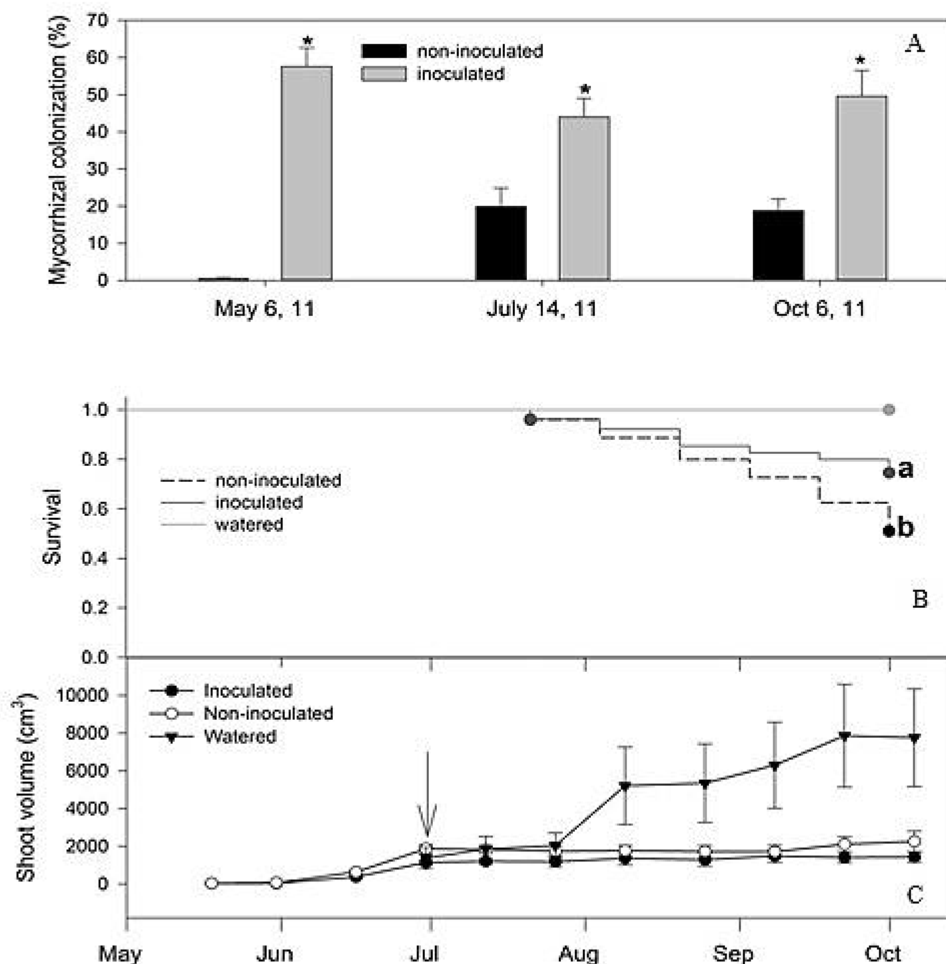

Figure 2. Mycorrhizal colonization (A), survival (B), and shoot growth (C) of sagebrush seedlings growing in pots at the Idaho Botanical Gardens. A, Mycorrhizal colonization at the time of transplanting (May 6) and two and five months afterwards. Each bar represents the mean (± SE) of at least five seedlings. 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 (± SE) of all living seedlings in a particular treatment. The arrow, on July 1, indicates the initiation of irrigation for the watered treatment.

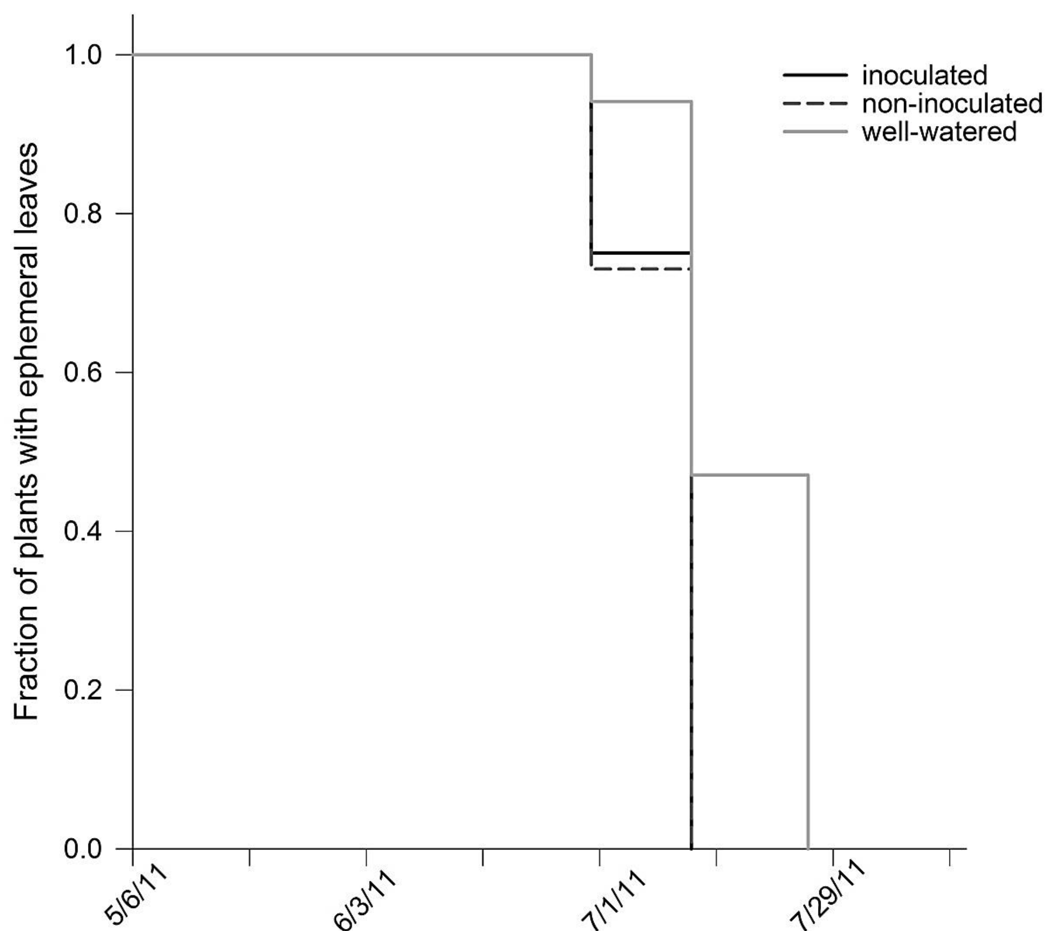

Figure 3. **Time course of ephemeral leaf senescence as measured by the fraction of plants with ephemeral leaves. Based on a log-rank test, drop of ephemeral leaves occurred later in well-watered plants than those experiencing natural precipitation (*p* < 0.001).**

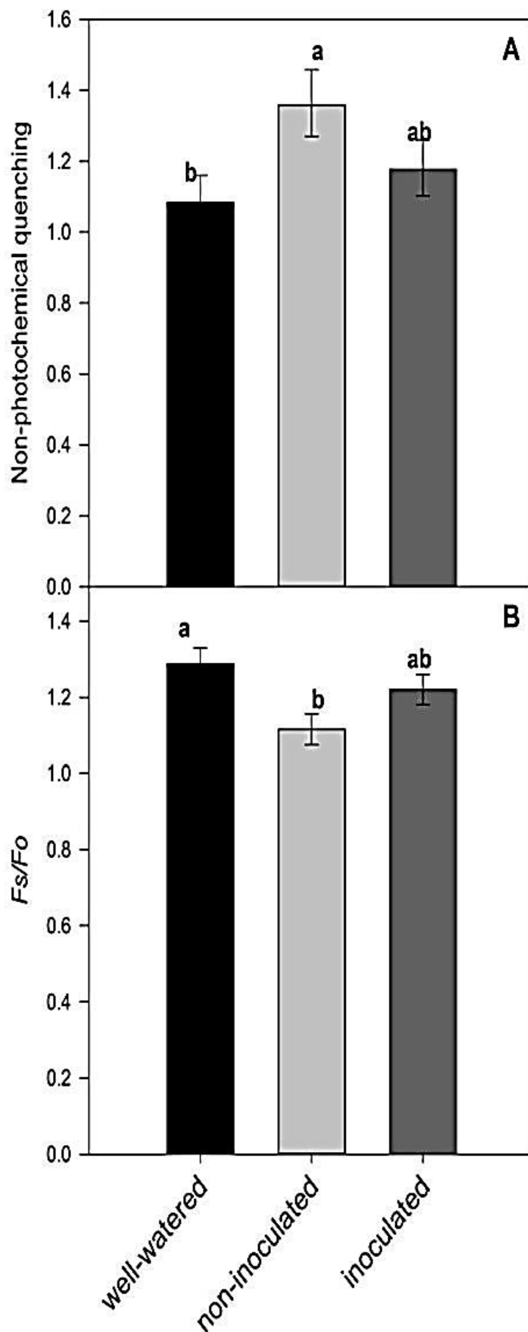

Figure 4. Chorophyll fluorescence parameters of *Artemisia tridentata* seedlings grown outdoors during the spring and summer of 2011. Non-photochemical quenching (A) and steady state fluorescence normalized to its dark-adapted state (*Fs/Fo*) (B) of well watered seedlings and of non-inoculated and inoculated 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.

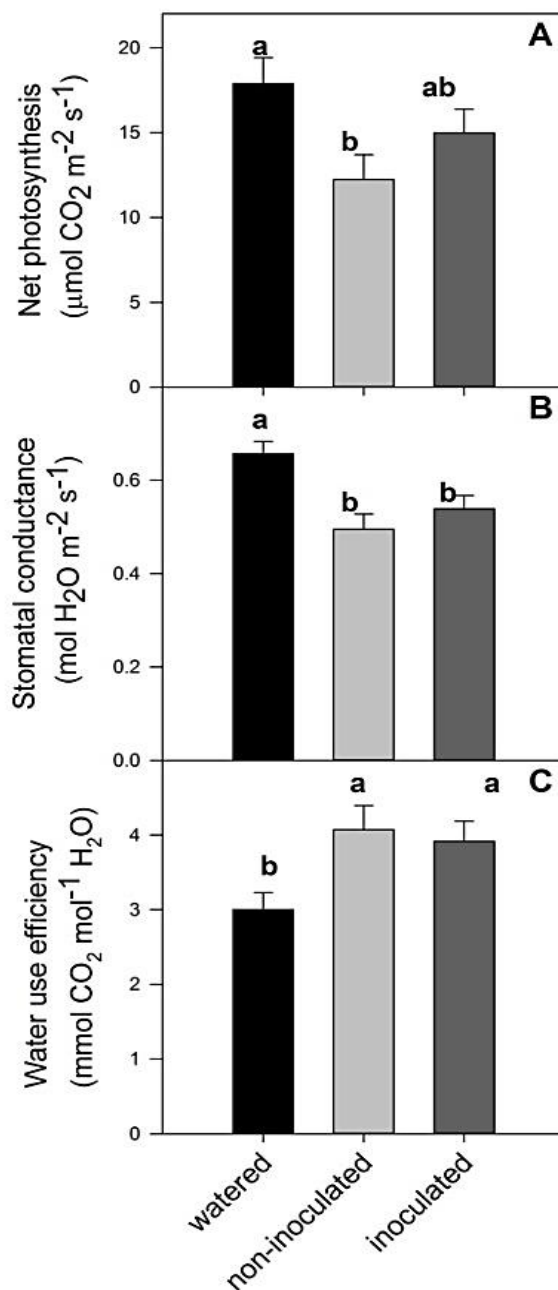

Figure 5. **Influence of watering and mycorrhizal inoculation on gas exchange parameters of *Artemisia tridentanta* seedlings grown outdoors during the spring and summer of 2011. Net photosynthesis (A), stomatal conductance (B), and photosynthetic water use efficiency (C) of well watered seedlings and 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.**

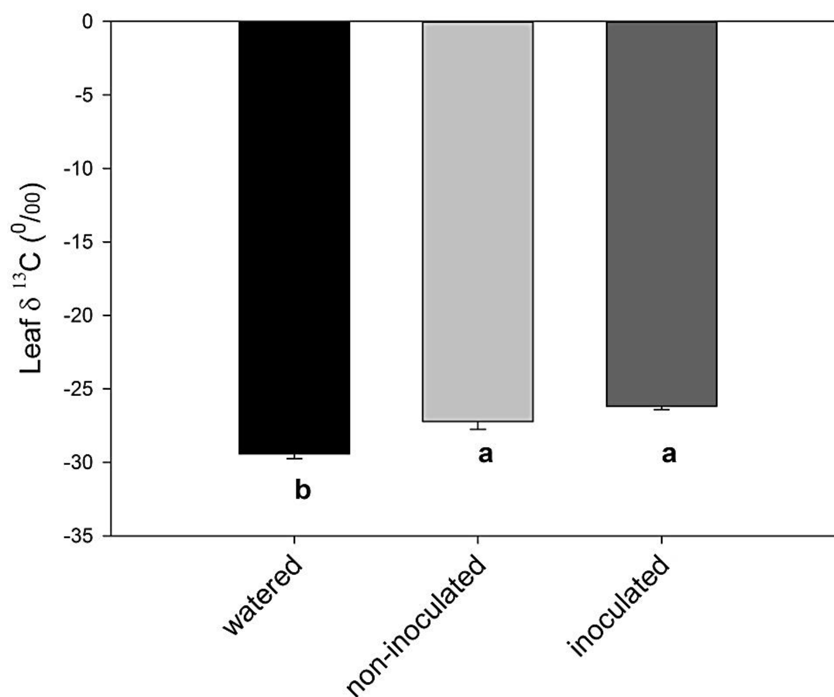

Figure 6. **Foliar carbon isotope ratios (δ ^{13}C) of *Artermisia tridentata* seedlings. Each bar represents the mean (-SE) of 5 seedlings. Leaves for δ ^{13}C analysis were collected on October 6, 2011, at the end of spring transplanting experiment. Means not labelled with the same letter are significantly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

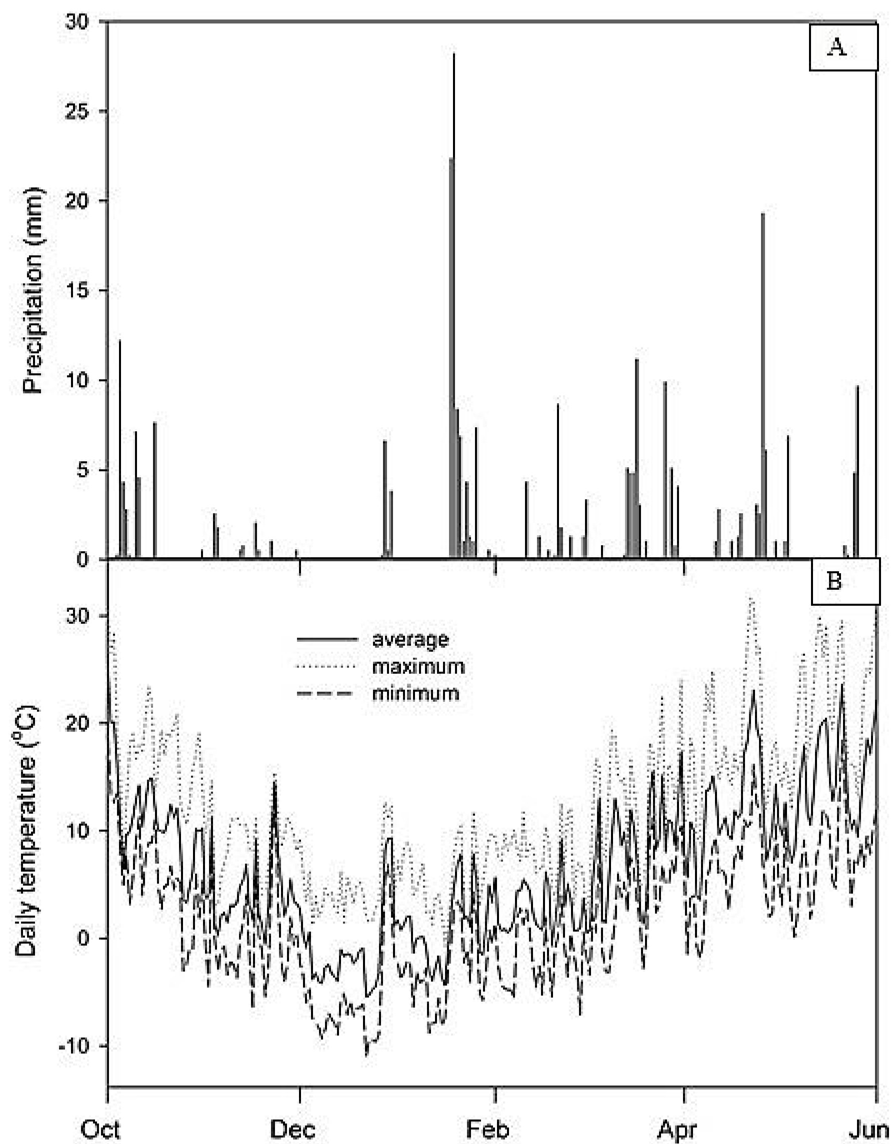

Figure 7. Weather conditions from October 1, 2011 to mid- June, 2012 at the Idaho Botanical Gardens experienced by seedlings in the fall mesocosm experiment. A, daily precipitation; B, Daily average, minimum, and maximum temperatures.

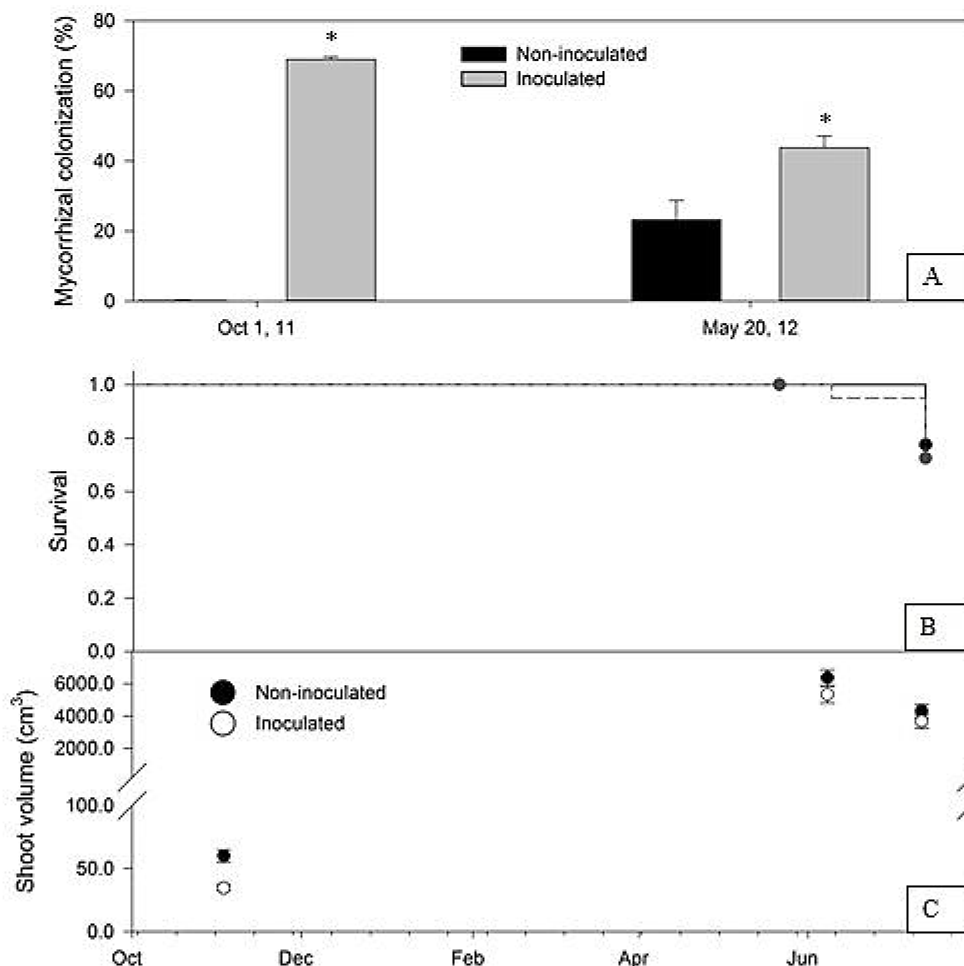

Figure 8. Mycorrhizal colonization (A), survival (B), and shoot growth (C) of sagebrush seedlings growing in pots at the Idaho Botanical Gardens from October 2011 to June 2012. A, Mycorrhizal colonization at the time of transplanting and seven and half months later. Each bar represents the mean (± SE) 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 (± 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.

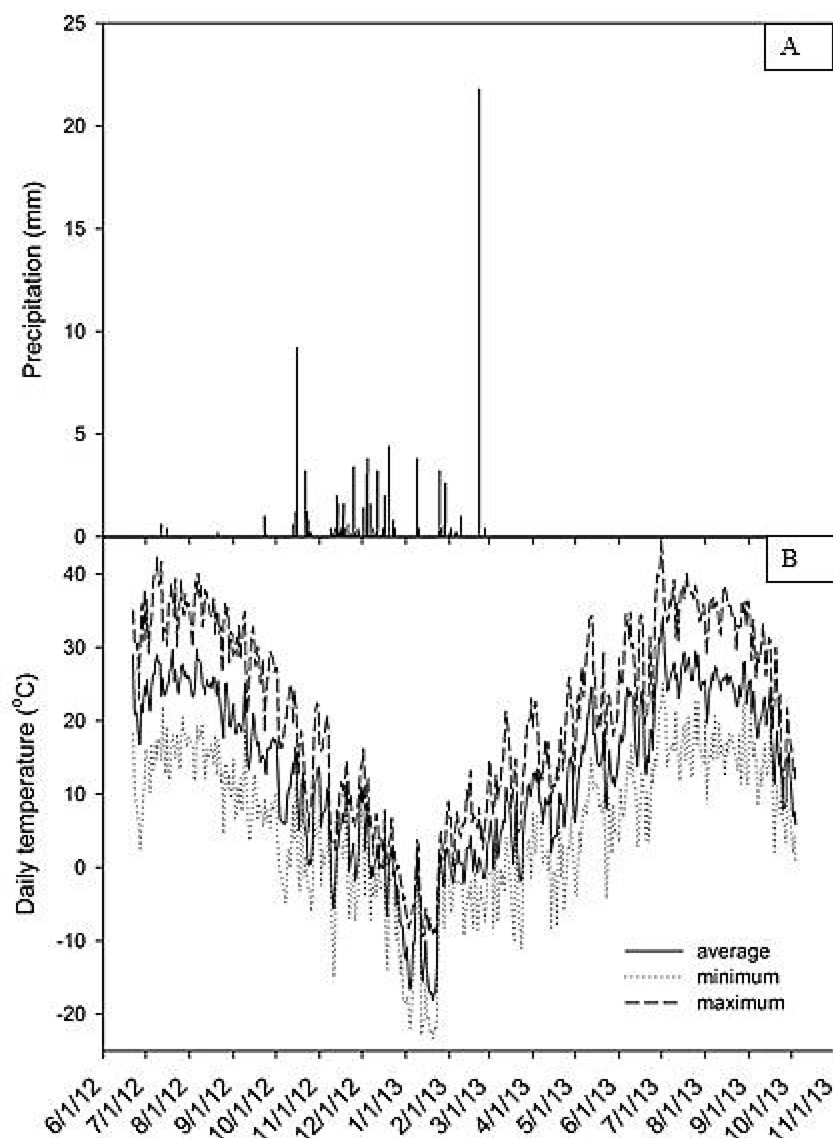

Figure 9. Weather conditions from June 1, 2012 to November 1, 2013 at the field site near Big Foot Butte, Snake River Birds of Prey, NCA. Panel (A) presents naturally occurring precipitation (mm) over the duration of the spring and fall 2012 field experiments. Panel (B): Temperature (°C) is depicted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

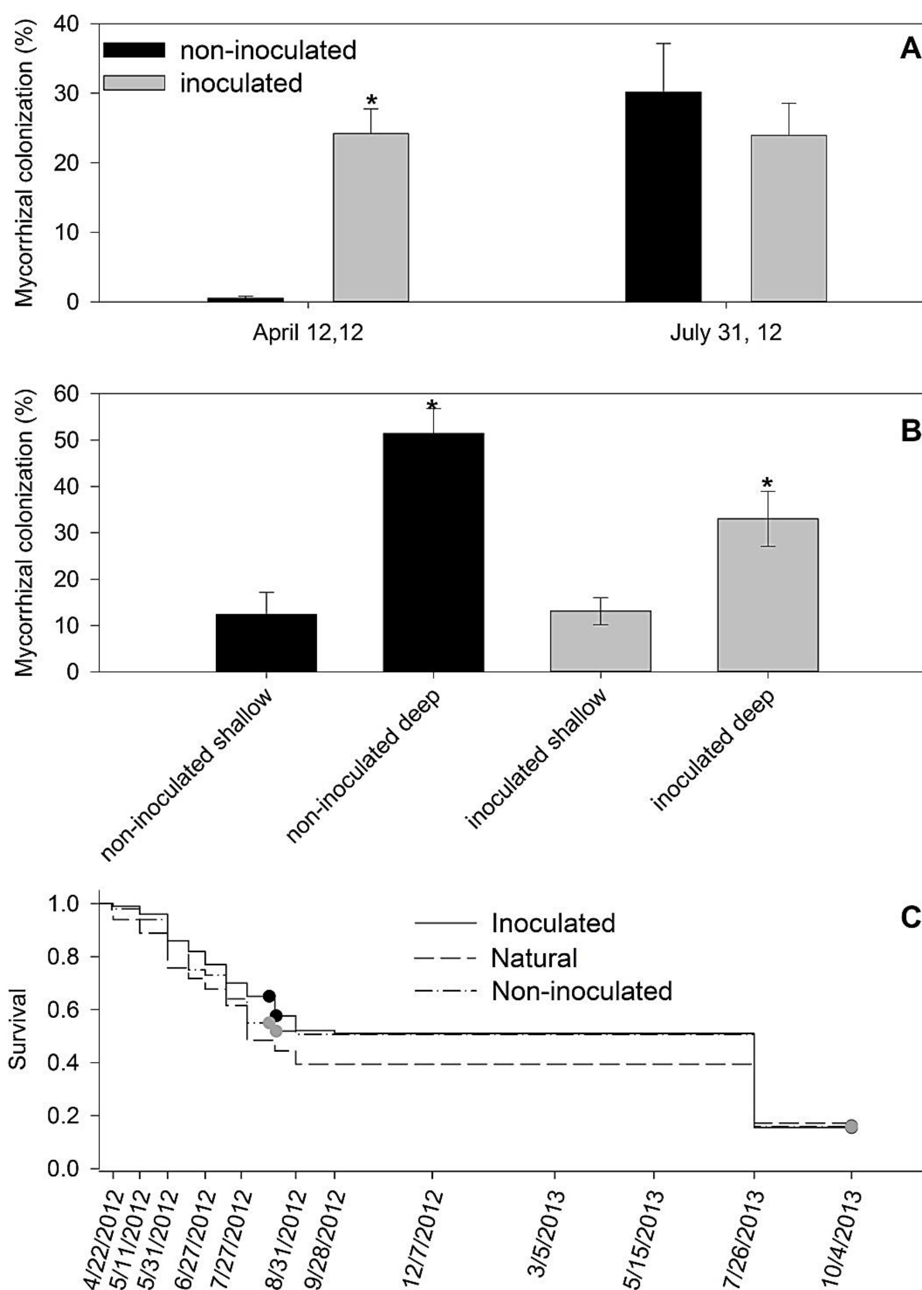

Figure 10. Mycorrhizal colonization by treatment (A) and rooting depth (B), and survival (C) of sagebrush seedlings transplanted into the Big Foot Butte site on April 2012. Each bar represents the mean (± SE) of six seedlings. In A, bars marked by an asterisk are significantly different from the non-inoculated 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.

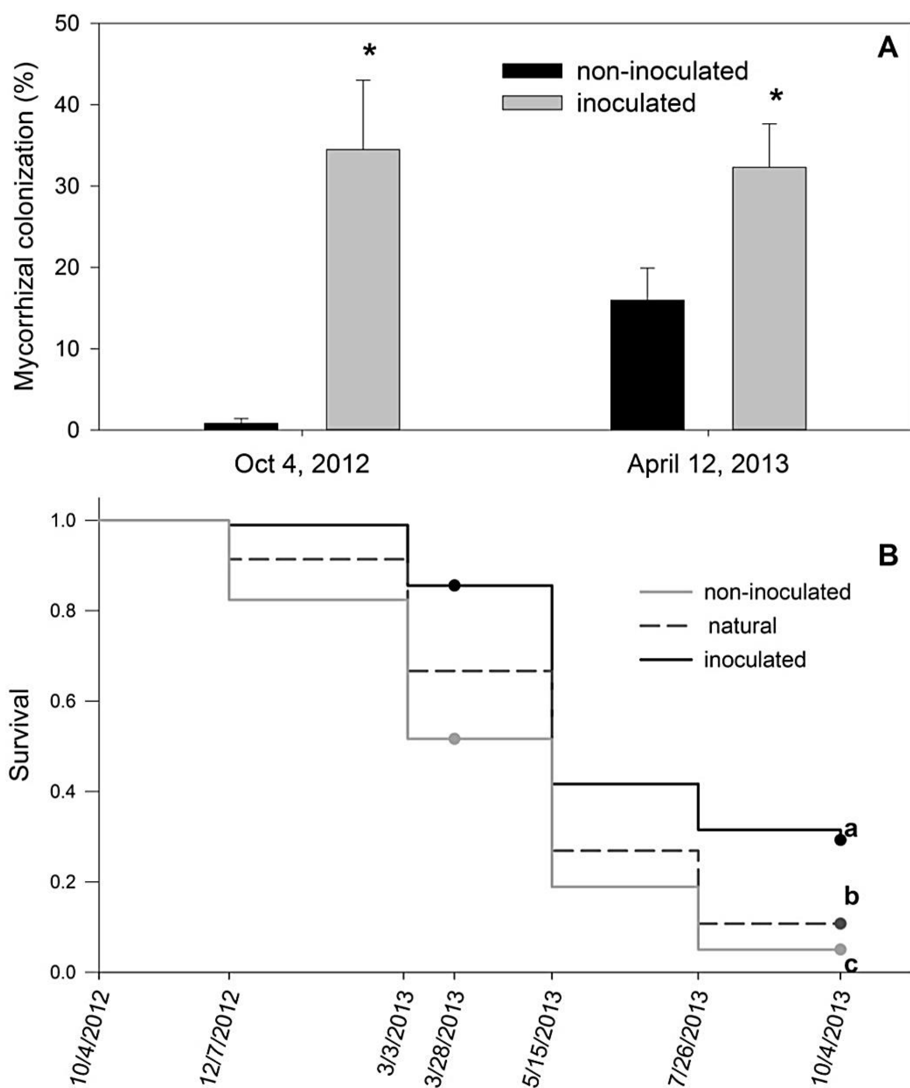

Figure 11. Mycorrhizal colonization (A) and survival (B) of sagebrush seedlings transplanted into the Big Foot Butte site on October 4, 2012. In A, each bar represents the mean (± SE) of six seedlings; bars marked by an asterisk are significantly different from the non-inoculated seedlings ($p < 0.05$).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

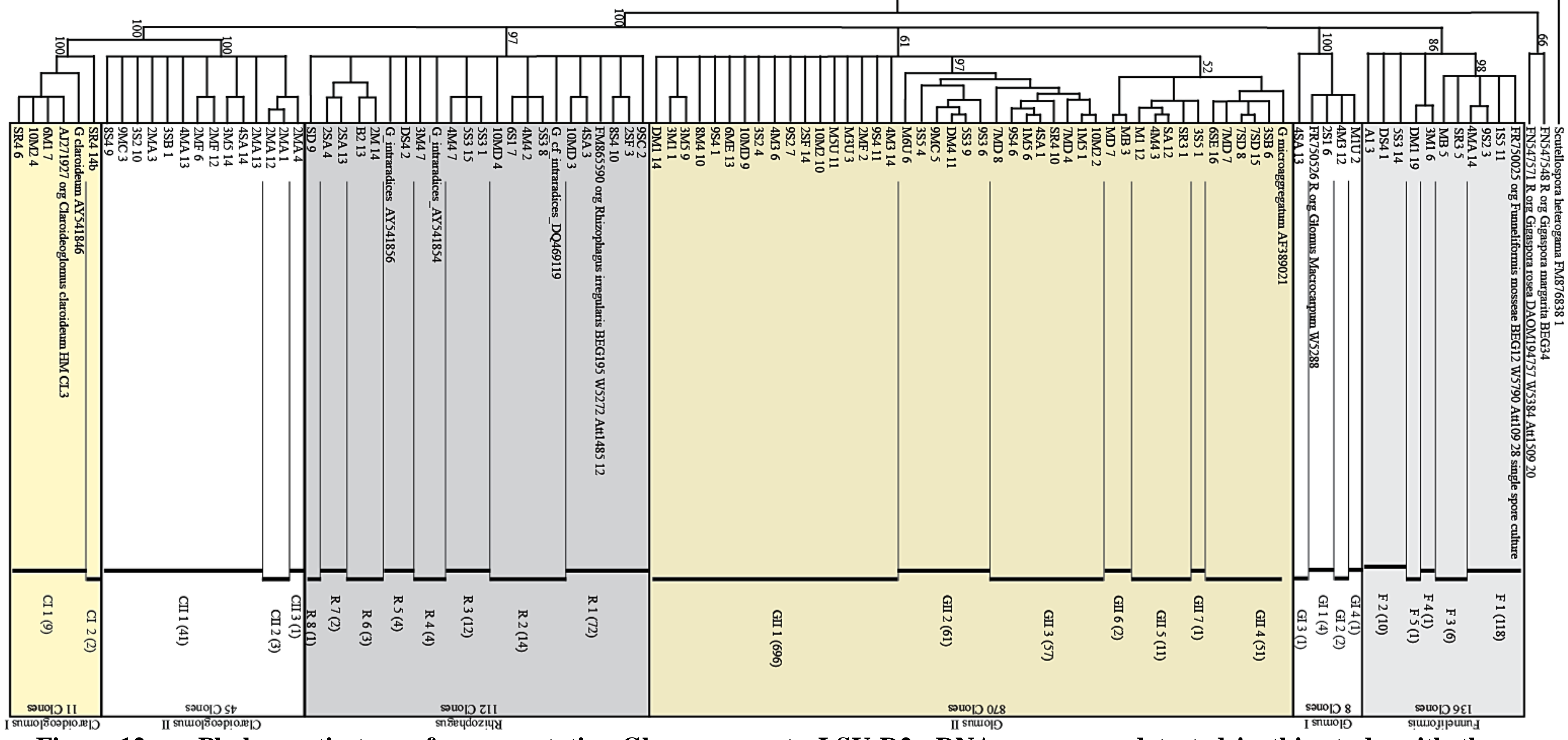

Figure 12. Phylogenetic tree of representative Glomeromycota LSU-D2 rDNA sequences detected in this study with the FLR3/FLR4 primers and database sequences of known Glomeromycota. Maximum Parsimony analysis with bootstrap support values above clade branches. Names and abundance of the six phylotypes identified are indicated on the right margin.

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.

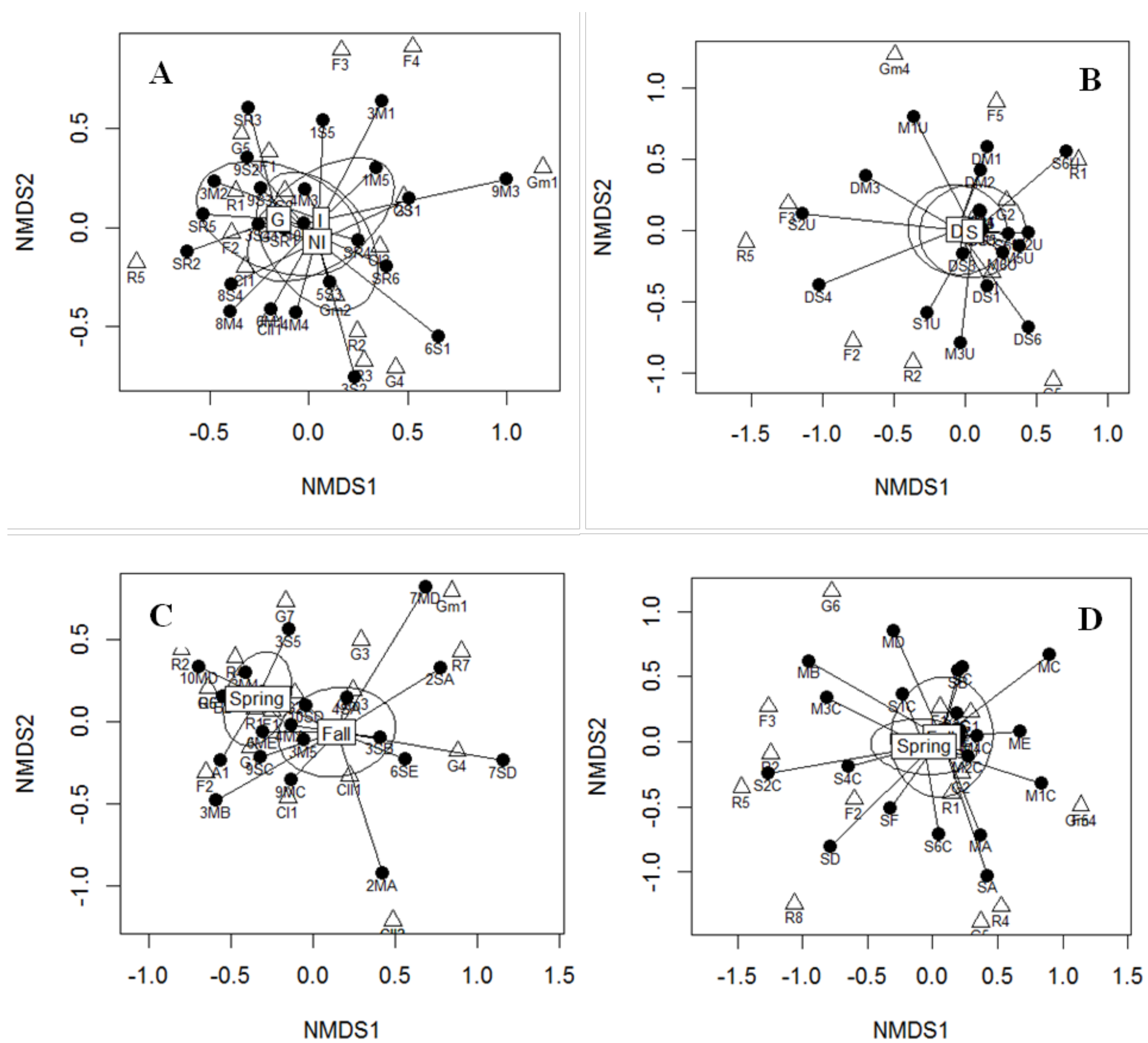

Figure 14. Analysis of arbuscular mycorrhizae communities by nonmetric multidimensional scaling. A, Comparison of AMF communities obtained from greenhouse-inoculated seedlings prior to transplanting (G) with those obtained from non-inoculated (NI) and inoculated (I) seedlings three months after transplanting 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 mesoscosm 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.

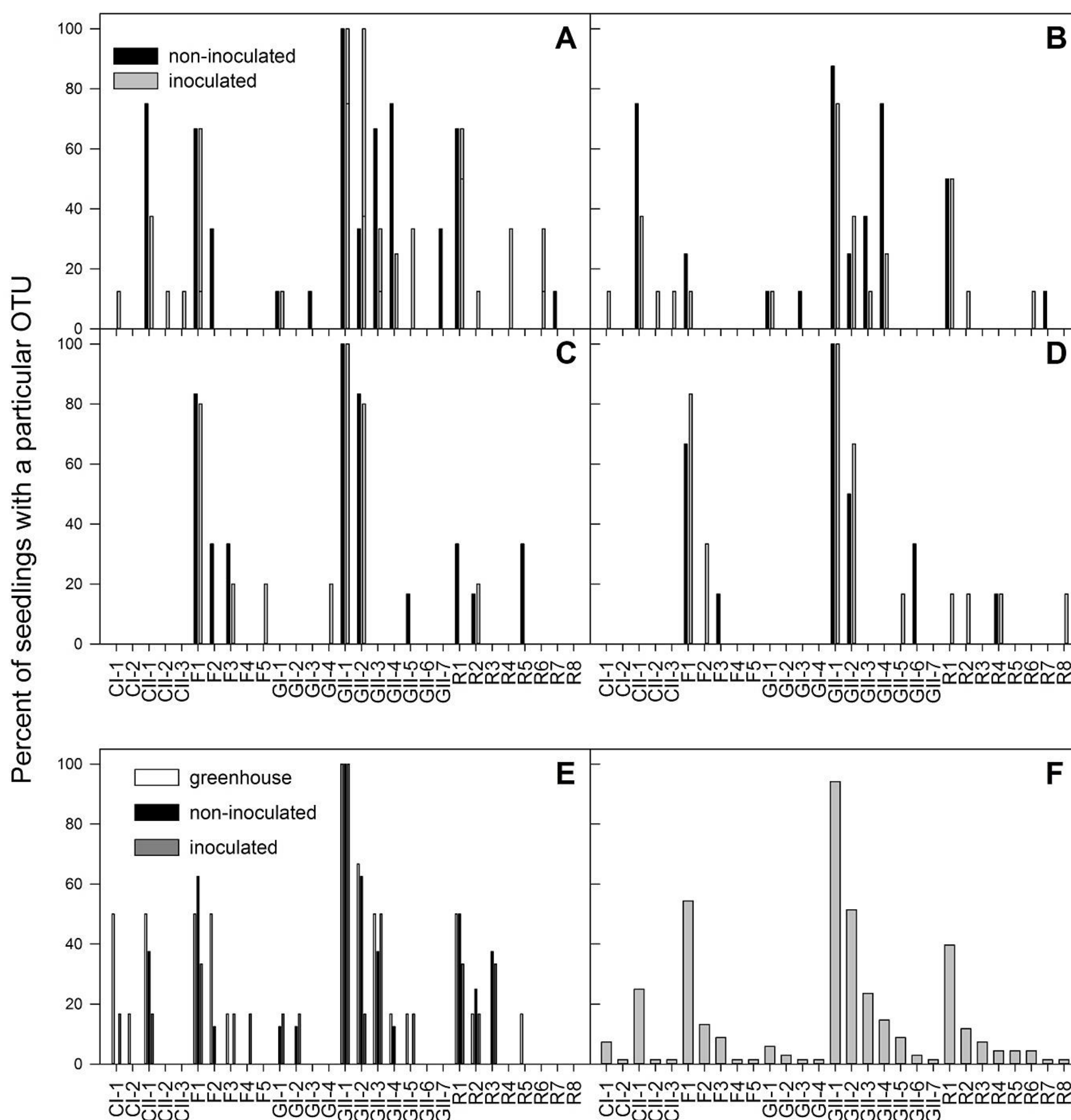

Figure 15. Frequency of different AMF OTUs found to be colonizing roots of *Artemisia tridentata* seedlings. Results from the spring 11 (A), fall 11 (B) and spring 12 (E) mesocosm experiments, and from the spring 12 (C) and fall 12 (D) field experiments. F, Overall frequencies observed after combining 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.