

Consequences of inoculation with native arbuscular mycorrhizal fungi for root colonization and survival of *Artemisia tridentata* ssp. *wyomingensis* seedlings after transplanting

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Abstract In arid environments, the propagule density of arbuscular mycorrhizal fungi (AMF) may limit the extent of the plant–AMF symbiosis. Inoculation of seedlings with AMF could alleviate this problem, but the success of this practice largely depends on the ability of the inoculum to multiply and colonize the growing root system after transplanting. These phenomena were investigated in *Artemisia tridentata* ssp. *wyomingensis* (Wyoming big sagebrush) seedlings inoculated with native AMF. Seedlings were first grown in a greenhouse in soil without AMF (non-inoculated seedlings) or with AMF (inoculated seedlings). In spring and fall, 3-month-old seedlings were transplanted outdoors to 24-L pots containing soil from a sagebrush habitat (spring and fall mesocosm experiments) or to a recently burned sagebrush habitat (spring and fall field experiments). Five or 8 months after transplanting, colonization was about twofold higher in inoculated than non-inoculated seedlings, except for the spring field experiment. In the mesocosm experiments, inoculation increased survival during the summer by 24 % ($p=0.011$). In the field experiments, increased AMF colonization was associated with increases in survival during cold and dry periods; 1 year after transplanting, survival of inoculated seedlings was 27 % higher than that of non-inoculated ones ($p<0.001$). To investigate possible mechanisms by which AMF increased survival, we analyzed water use efficiency (WUE) based on foliar

$^{13}\text{C}/^{12}\text{C}$ isotope ratios ($\delta^{13}\text{C}$). A positive correlation between AMF colonization and $\delta^{13}\text{C}$ values was observed in the spring mesocosm experiment. In contrast, inoculation did not affect the $\delta^{13}\text{C}$ values of fall transplanted seedlings that were collected the subsequent spring. The effectiveness of AMF inoculation on enhancing colonization and reducing seedling mortality varied among the different experiments, but average effects were estimated by meta-analyses. Several months after transplanting, average AMF colonization was in proportion 84 % higher in inoculated than non-inoculated seedlings ($p=0.0042$), while the average risk of seedling mortality was 42 % lower in inoculated than non-inoculated seedlings ($p=0.047$). These results indicate that inoculation can increase AMF colonization over the background levels occurring in the soil, leading to higher rates of survival.

Keywords Arbuscular mycorrhizal fungi · *Artemisia tridentata* · Seedling establishment · Sagebrush steppe · Shrublands

Introduction

Arbuscular mycorrhizal fungi (AMF) are obligate biotrophs that obtain organic carbon from the host plant and, in return, facilitate nutrient uptake. Various studies also indicate that AMF colonization can increase plant drought tolerance (Doubkova et al. 2013; Jayne and Quigley 2014). Several processes may contribute to this effect. As soil moisture declines, nutrient uptake becomes more limiting due to the lengthening of the pathway for nutrient movement to the root surface (Sardans and Penuelas 2007; Suriyagoda et al. 2014). This limitation is particularly severe for phosphorus and other nutrients with low mobility in the soil (Suriyagoda et al. 2014). Thus, as soil moisture declines, the presence of AMF

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becomes more crucial for P uptake (Augé 2001; Suriyagoda et al. 2014). In addition, the extra-radical hyphae of AMF may contribute to the maintenance of liquid continuity through soil air gaps, thus facilitating movement of water towards the roots (Augé 2001). However, the extent of this effect may be low and it depends on the density of extra-radical hyphae and soil drying patterns (Augé et al. 2003; Allen 2007).

Most studies on the effects of AMF on plants have been based on comparisons of non-AMF and AMF-plants, a situation that rarely occurs in nature (Allen 2007; Paluch et al. 2013). Under field conditions, most plants are colonized to some extent by AMF. However, it is unclear whether the natural levels of AMF colonization are sufficient to optimize plant survival and growth. In semiarid and arid regions, in particular, AMF spore density in the soil can be low and AMF colonization of roots may be largely mediated by an established hyphal network that maintains infectivity (Wicklow-Howard 1989; Pattinson et al. 1999; Allen et al. 2003). The establishment of plant–AMF symbioses by this hyphal network may be disrupted by changes in vegetation composition and disturbances such as fires (Vilarino and Arines 1991; Mummey and Rillig 2006; O’Dea 2007). Under this scenario, practices that increase AMF propagule density such as inoculation of seedlings before or at the time of transplanting can be beneficial for restoration of native habitats (Turnau and Haselwandter 2002; Allen et al. 2005; Navarro Garcia et al. 2011).

A common semiarid habitat in western North America is the sagebrush steppe. This habitat covers approximately 450,000 km² and is characterized by a vegetative community composed of perennial grasses, forbs, biological soil crusts, and several subspecies of the shrub *Artemisia tridentata* Nutt (big sagebrush) (Noss et al. 1995; Anderson and Inouye 2001). Over the past century, sagebrush habitats have been disturbed by overgrazing and invasion by exotic grasses (Noss et al. 1995; Brooks et al. 2004). In particular, invasion by *Bromus tectorum* has disproportionately affected sagebrush communities by decreasing the fire return interval, which tends to eliminate *A. tridentata* (D’Antonio and Vitousek 1992; Anderson and Inouye 2001; Brooks et al. 2004). *A. tridentata* plays important ecological roles by contributing to the development of a heterogeneous landscape and providing habitat and forage for local animals including greater sage-grouse (*Centrocercus urophasianus*) and pigmy rabbit (*Brachylagus idahoensis*), which depend on *A. tridentata* for most of their winter diet (Charley and West 1977; Aldridge and Boyce 2007; Davies et al. 2007; Larrucea and Brussard 2008). Due to the critical functions of *A. tridentata* in sagebrush habitats, there is considerable interest in re-establishing this species following fires and in particular one of the subspecies of *A. tridentata*, ssp. *wyomingensis* Beetle & Young (Wyoming big sagebrush) (Cox and Anderson 2004; Boyd and Obradovich 2014). This subspecies occurs throughout western North America,

where it usually occupies xeric locations that receive 200–300 mm of annual precipitation (Perryman et al. 2001). These environmental conditions make re-establishment of *A. tridentata* ssp. *wyomingensis* particularly difficult (Cox and Anderson 2004; Boyd and Obradovich 2014). A major factor responsible for low establishment is high seedling mortality during summer drought (Stahl et al. 1998; Dalzell 2004).

In sagebrush steppe ecosystems, summer precipitation is rare and moisture is progressively limited to deeper portions of the soil (Hacke et al. 2000). Under these conditions, sagebrush seedlings benefit from plant responses that lead to a more efficient use of the available water and/or an increase in the ability to extract water from deeper, moister soil (Hacke et al. 2000). AMF can increase water use efficiency (WUE) via an increase in photosynthetic capacity as result of improved nutrition and the additional carbon demand needed to sustain fungal hyphae (Querejeta et al. 2003). In addition, AMF tend to increase stomatal conductance (Augé et al. 2014). Although this effect increases water loss and reduces WUE, the plant may gain more carbon for root and hyphal growth (Wu and Zou 2010; Navarro Garcia et al. 2011). Thus, higher photosynthetic capacity and stomatal conductance may, through an effect on growth, allow mycorrhizal roots to reach deeper regions of the soil.

Independent of the possible mechanisms involved, an AMF-induced increase in drought tolerance would tend to enhance establishment of Wyoming big sagebrush seedlings (Stahl et al. 1998). Presently, however, the extent to which AMF can increase drought tolerance and establishment of these seedlings is uncertain. From a restoration perspective, an additional question is whether the density of the resident AMF propagules is sufficient to optimize colonization and seedling establishment or whether it can be improved via inoculation. To investigate these questions, we conducted mesocosm and field experiments. The mesocosm experiments involved transplanting non-inoculated seedlings and seedlings inoculated with native AMF to pots filled with soil from a sagebrush habitat, while field experiments involved transplanting to a recently burned sagebrush site. Subsequently, we analyzed the effect of inoculation on colonization and seedling survival after transplanting. Native AMF were used because they are adapted to local climatic and edaphic conditions and thus are more likely to survive and propagate after transplanting than non-native AMF (Weinbaum et al. 1996; Requena et al. 2001; Rowe et al. 2007). In addition, the ecological consequences of introducing AMF are poorly understood (Schwartz et al. 2006; Symanczik et al. 2015). This limited understanding is partly attributed to the complex nature of the AMF–plant symbiosis, which can range from mutualistic to parasitic, depending on environmental conditions and the particular AMF and plant

genotypes involved in the association (Klironomos 2003; Jones and Smith 2004; Johnson and Graham 2013). By using native AMF, we sought to reduce the chances of introducing taxa that could negatively affect sagebrush seedlings.

In western North America, transplanting of Wyoming big sagebrush has been conducted either during mid-spring (April to mid-May) or in early fall (October). After spring transplanting, drought tends to be the main limiting factor for seedling growth and establishment. In contrast, following fall, transplanting seedling growth tends to be most limited by low temperatures. Differences in moisture and temperature may affect AMF growth after transplanting as well as AMF effects on seedling establishment (Allen 1983; Trent et al. 1994; Santos-Gonzalez et al. 2007). To investigate these possibilities, we conducted transplanting experiments in both spring and fall.

As discussed above, AMF can potentially increase establishment through several mechanisms. If AMF inoculation were to increase seedling survival, we hypothesized that an insight into the mechanisms responsible for this increase could be gained by analyzing the foliar $^{13}\text{C}/^{12}\text{C}$ isotope ratio ($\delta^{13}\text{C}$). Values of $\delta^{13}\text{C}$ provide an indirect, but integrative estimate of the ratio of net photosynthesis over stomatal conductance through the growing season and, thus, a relative estimate of intrinsic water use efficiency (Donovan and Ehleringer 1994). An increase in WUE would be expected to occur as a result of an AMF-induced increase in photosynthetic capacity, while a decrease would tend to occur if AMF increased stomatal conductance. Clearly, these two changes could neutralize each other resulting in no alteration in WUE. To distinguish this possibility, we also evaluated in one of the experiments WUE based on instantaneous measurements of photosynthesis and stomatal conductance over the summer. These results were complemented with measurements of plant biomass, which would tend to increase with enhancements in photosynthetic capacity.

Taken together, the general hypothesis of this study was that inoculation would increase colonization over the levels caused by the resident AMF community and that this increase would increase seedling establishment. Within this context, our aims were to answer the following questions: Can the background levels of AMF colonization be increased by inoculation with native AMF? Are the effects of inoculation on AMF colonization and seedling survival different during spring and fall transplanting? Are increases in colonization associated with changes in WUE and seedling survival? We anticipated that answering these questions would increase knowledge of AMF ecology in sagebrush habitats and may also lead to improved techniques for the reestablishment of Wyoming big sagebrush following disturbances.

Materials and methods

Plant and fungal material

Wyoming big sagebrush seeds were collected near Casper, Wyoming (42° 30' N, 106° 30' W) and near Big Foot Butte, Idaho (43° 18' 48.43" N, 116° 21' 48.57" W). Seeds collected near Casper-Wyoming were exclusively used for the mesocosm experiments, while those collected near Big Foot Butte-Idaho exclusively used in the field experiments. To produce mycorrhizal inoculum, sandy soil was collected from Kuna Butte, Idaho (43° 26.161' N, 116° 25.848' W, 908 m a.s.l.), a sagebrush-steppe community south of Boise, Idaho. Pot cultures were prepared as previously described (Carter et al. 2014) using Sudan grass (*Sorghum bicolor* L. var. *sudanense*) as a host. After three cycles of pot culture cultivation, the soil and roots were stored in plastic bags and within 4 months used to inoculate sagebrush seedlings. The AMF taxa present in the roots of inoculated sagebrush seedlings prior to transplanting were determined based on sequences from the D2 region of the 28S ribosomal RNA gene (LSU-D2) as described by Carter et al. (2014). The phylogenetic analysis of these sequences revealed sequences that clustered with *Rhizophagus intraradices*, *Glomus microaggregatum*, *Funneliformis mosseae*, *Claroideoglomus claroideum*, or with published sequences of uncultured and unnamed glomeromycetes within the *Glomus* genus (Mummey and Rillig 2007; Torrecillas et al. 2012; Carter et al. 2014). The sequences generated have been deposited at the National Center for Biotechnology Information (NCBI) GenBank (<http://www.ncbi.nlm.nih.gov/>) under the accession numbers KR819280 - KR819395.

Soil chemical and physical analysis

The soil used in the mesocosm experiments was collected at Kuna Butte, while the field experiments were conducted at the Big Foot site. For each site, equal volumes of soil from five subplots were thoroughly mixed and an aliquot of this mixture was sent for analysis to the University of Idaho Analytical Sciences Laboratory. Results of these analyses are summarized in Table 1. The soil collected at Kuna Butte was sandy and had an available P content of 8.1 $\mu\text{g g}^{-1}$ of soil (Table 1). In contrast, the Big Foot field site has a silty-loam soil, which had an available phosphorus content of 43 $\mu\text{g g}^{-1}$ of soil. P in both soils was estimated based on bicarbonate extraction and subsequent assessment by the Olsen method.

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 (inoculated seedlings) or sterilized pot cultures

Table 1 Nutrient, pH, and textural characteristics of the soils used for the mesocosm and field experiments. Nutrients are expressed in $\mu\text{g g}^{-1}$ of dry soil, and organic matter and soil particles in percent weight by weight

Experiment	K	P	$\text{NO}_3^- + \text{NO}_2^-$	NH_4^+	Organic matter	pH	Sand %	Clay %	Silt %	Texture
Mesocosm	135	8.1	45	1.9	0.6	7.5	88.8	5	6.2	Sand
Field	620	43	16	2.2	2.1	7.5	36.2	8.8	55	Silt loam

supplemented with an AMF-free microbial wash (non-inoculated seedlings). This wash was obtained from the pot cultures prior to autoclaving as described by Mummey et al. (2009). After 3 months, the seedlings were transplanted to 24-L tree pots (78.8 cm in height and 20 cm on average diameter), which were filled with untreated soil from Kuna Butte and placed above ground. Two experiments were conducted. For the spring transplanting experiment, the seedlings were transplanted in May of 2011 and for the fall transplanting experiment in October of the same year. For the spring transplanting experiment, 150 pots were used, half with non-inoculated seedlings and half with inoculated ones. The fall transplanting experiment followed a similar approach; however, 50 pots were prepared per treatment. After transplanting, plants were grown outdoors under ambient temperature and precipitation conditions at the Idaho Botanical Gardens (N 43° 35' 52.1" W 116° 9' 42.3", 851 m), which is located within an *A. tridentata* ecosystem. Pots were distributed at random, and a weather station at the experimental site recorded temperature and precipitation. The temperature and precipitation collected at the site were compared with average values from 1981 to 2010 obtained from the Boise Airport weather station (N 43° 33' 51.8394", W 116° 13' 22.08", 865 m, <http://www.wrcc.dri.edu/>).

To assess soil water moisture and plant water status, we conducted measurements of soil volumetric water content and predawn water potential, respectively, but only for the spring transplanting experiment. Soil volumetric water content was monitored hourly with moisture probes (Echo 20; Decagon Devices, Inc., Pullman, WA) in 5 pots per treatment. The probes were placed at about 30 cm from the soil surface until mid-July 2011 and subsequently were moved to a depth of about 70 cm. Predawn water potentials were measured using a Scholander pressure chamber (PMS Instrument Company). These measurements were taken on 5 seedlings per treatment and only once at the beginning of September because accurate measurements of water potential required destructive sampling of seedlings.

During the spring transplanting experiment, seedling survival was measured biweekly and AMF colonization was measured just prior to transplanting and 2.5 and 5 months afterwards. In the fall transplanting experiment, AMF colonization was determined prior to transplanting and 7 months afterwards, and survival at monthly intervals. For analysis of

AMF colonization, six seedlings per treatment were selected at random and their whole, intact, root system harvested. Based on preliminary measurements, this sampling effort was sufficient to detect statistical differences in colonization when the average difference was about 20 %. We hypothesized that a difference smaller than 20 % might not be sufficient to affect survival. This notion was based on results obtained with Wyoming big sagebrush seedlings inoculated with a commercial inoculum (Gurr and Wicklow-Howard 1994). In this study, inoculation increased colonization between 10 and 20 %, but did not affect survival under field conditions. For the analysis of colonization, the whole root system was harvested rather than clipping roots segments from roots in pots because both approaches were equally destructive, but the former procedure facilitated the collection of fine roots. Approximately, 100 root segments, each 1 to 2 cm in length, were sampled at random from portions of the root system that lacked secondary growth. The root segments were placed in 5 % KOH and cleared at 121 °C for 5 min. Subsequently, the root segments were rinsed with water, immersed in 1 % hydrochloric acid (HCl) for 20 min, and then stained overnight with 0.03 % Chlorazol Black E in lactoglycerol (1:1:1 water/glycerin/lactic acid). Roots were de-stained for 1 to 2 min with dimethyl sulfoxide (DMSO) to reduce non-specific staining, rinsed with water, and stored and mounted in 50 % glycerol. Total mycorrhizal and arbuscular colonization were estimated by the intersection method using 200 intersections per sample (McGonigle et al. 1990). Any mycorrhizal structure crossing the intersection was counted to estimate total AMF colonization, while only arbuscules were counted for arbuscular colonization. At the end of the experiment, the plants used to estimate colonization (six per treatment) were also used to measure dry biomass and the shoot over root ratio. For these measurements, shoots and roots were dried at 80 °C until no changes in weight were detected between successive days.

Leaves from the seedlings used to measure biomass were also sampled to estimate intrinsic WUE based on the foliar $^{13}\text{C}/^{12}\text{C}$ isotope ratio ($\delta^{13}\text{C}$). For the $\delta^{13}\text{C}$ analysis, dry leaves were ground using copper plated balls (H&N sport 4.50 mm BB). Subsequently, samples were processed using a Costech 3140 flash combustion gas chromatograph (Costech Analytical Technologies Inc.) that delivered the samples for isotope analysis to a Picarro 2020 CRDS laser spectrometer (Picarro Inc.). Analytical precision and isotope

values were calculated following standard methods as described by Richardson et al. (2014).

WUE was also estimated by gas exchange measurements, but only for the spring transplanting experiment. For this purpose, five plants per treatment were selected at random every day of measurements and leaves were placed in a LI-6400-40 chamber connected to a LI-COR 6400XT portable photosynthesis system (LI-COR Inc.). For each sample, net photosynthesis, stomatal conductance, transpiration, and WUE were determined. These parameters were measured at an incoming air CO₂ concentration of 400 $\mu\text{mol mol}^{-1}$, 50 (± 5) % relative humidity, ambient temperature, and a saturating light intensity of 1700 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Leaves were arranged to fully cover the leaf chamber, and values of net photosynthesis and transpiration were recorded after the CO₂ assimilation rates and stomatal conductance values became stable. Measurements of gas exchange were made bi-weekly from June to October 2011.

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, 863 m), within the Morely Nelson Snake River Birds of Prey, National Conservation Area. The site had experienced a fire during the previous summer that killed all the sagebrush plants. For the field experiments, we initially grew sagebrush seedlings for 3 months in the greenhouse, but instead of using cone-tainers, we used Jiffy-7 peat pellets (Jiffy Products Ltd.). These pellets facilitated field transplanting, and preliminary experiments indicated that they could be used to pre-inoculate seedlings with AMF. The center of the pellet was filled with either 6 ml of sterile soil (non-inoculated treatment), or soil and roots from the Sudan grass pot cultures of native mycorrhizae (inoculated treatment). One hundred seedlings were transplanted per treatment for a total of 200 seedlings on April 12, 2012 (spring transplanting) and another total of 200 seedlings on October 4, 2012 (fall transplanting). Seedlings were transplanted within the Jiffy pellets at a distance of at least 5 m from each other and at about 1 m north-east from residual sagebrush stumps; this placement is believed to shade seedlings during the afternoon. Within these constraints, seedlings were distributed at random over 3.1 ha. After transplanting, seedlings were enclosed by a browsing protector tube (25.4 mm plastic mesh, 44 cm height, and 10 cm in diameter). A weather station located at the site recorded temperature and precipitation data that were compared with average values of temperature and precipitation from 2002 to 2011. Averages were calculated based on data recorded at the two most proximal Agrimet stations (Agrimet Weather Data, <http://www.usbr.gov/pn/agrimet/>, accessed May 1, 2015), one in Boise (N 43° 36' 0.972", W 116.17694, 831 m) and the other in Nampa (N 43° 26' 13.992", W 116° 38' 42.9714", 826 m).

For measurements of AMF colonization, seedlings were collected 4 and 7 months after the spring and fall transplanting, respectively. Per treatment and sampling time, six seedlings were selected at random and collected by carefully excavating the soil around the seedlings to retain the intact root system. For this purpose, a circle of approximately 0.75 m in radius was initially dug around the seedlings, but this radius gradually narrowed as the soil was excavated to a depth of about 1 or 0.5 m for plants collected during the summer (spring transplanting) and spring (fall transplanting), respectively. During these steps, particular care was taken to minimize damage to fine roots, where most of the AMF colonization occurs. Samples were stored at -24°C until processed. Shoots from the same seedlings for which root colonization was determined were used to measure shoot biomass and foliar $\delta^{13}\text{C}$. Methods for estimating colonization, shoot biomass, and foliar $\delta^{13}\text{C}$ were identical to those described earlier. Due to the small size of the seedlings, most of the corticated roots collected were used to analyze AMF-colonization.

Data analysis

For two-level analyses such as the comparison of AMF colonization, biomass, and $\delta^{13}\text{C}$ between non-inoculated and inoculated seedlings, the data were analyzed using an independent *t* test or a Welch *t* test if variances were equal or unequal, respectively. Some of the data on arbuscular colonization was not normally distributed; in these cases, comparisons between non-inoculated and inoculated seedlings were conducted using a Wilcoxon rank-sum test and results reported as medians rather than averages. The effect of inoculation, week of measurement, and the interaction between these factors on gas exchange parameters was analyzed using a mixed model with inoculation as a fixed factor and week of measurement and the interaction between inoculation and week of measurement as random factors. 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 stomatal conductance were not normally distributed; these values were Box-Cox transformed to generate a normal distribution. The significance of inoculation on gas exchange parameters was analyzed using a likelihood ratio test to compare a model without the inoculation factor with that of a model with inoculation as a factor. Differences in survival among treatments were evaluated using a logrank test. In addition to analyzing the effect of AMF inoculation on colonization, $\delta^{13}\text{C}$, and survival in individual experiments, meta-analyses were conducted to assess average effects across experiments. For this purpose, we used a random model because factors such as differences in weather, seed source, and soil characteristics could have influenced the effect of inoculation on the dependent variables (Hunter and Schmidt 2000). Within each study,

the effect size of inoculation on AMF-colonization and $\delta^{13}\text{C}$ was estimated as the natural log of the response ratio (RR), which was the mean of the inoculated seedlings divided by the mean of the non-inoculated ones (Hedges et al. 1999). The variance within each study was the standard error of $\ln$ RR. The effect size and its variance were calculated using the function *escalc* in the metaphor package in R (Viechtbauer 2010; R-Development-Core-Team 2013). For the meta-analysis of survival, the effect size and its variance were estimated as the natural log hazard ratio (HR) between inoculated and non-inoculated seedlings and the standard error of $\ln$ HR. Values for these parameters were obtained from the results of the Cox Proportional Hazard model used for the survival analysis. For meta-analysis of survival, only experiments were seedling mortality occurred were included. Survival analysis is based on known times at which an event of interest occurs, in our studies the death of a seedling; subjects that survive to the end of the experiment have an unknown survival time and therefore are censored from the analysis (Rich et al. 2010). All statistical analyses were conducted in R (R-Development-Core-Team 2013). Within this program, packages lme4, survival, and metafor were used to conduct the mixed model, survival analyses, and meta-analyses, respectively. Significant differences among treatments were determined at $p < 0.05$. All estimates of treatment variability are reported as standard errors.

Results

Spring transplanting mesocosm experiment

During the first 3 months of the experiment, temperatures were about 2 °C lower than normal. In contrast, they were about 2 °C higher than average in August and September (see Supplementary documents, Fig. 1a). In May and early June, precipitation maintained the soil at volumetric water contents of about 20 % (see Supplementary documents, Fig. 1b), which were similar to the volumetric water contents recorded in well-watered pots after drainage of excess water (data not shown). Subsequently, soil moisture began to decline; after late-August, the volumetric water content of the soil remained below 5 % and no difference in soil water content was detected between pots containing non-inoculated versus inoculated seedlings.

At the time of transplanting, AMF colonization of non-inoculated seedlings was negligible, while that of inoculated seedlings was 57.6 (± 7.08) % (Fig. 1a, $p < 0.001$). Albeit much smaller, differences were also noted for arbuscular colonization, with average values of 0.0 and 6.7 (± 2.8) % for non-inoculated and inoculated seedlings, respectively ($p = 0.03$). Two and a half months after transplanting, total colonization of non-inoculated seedlings was 25 % lower than that of

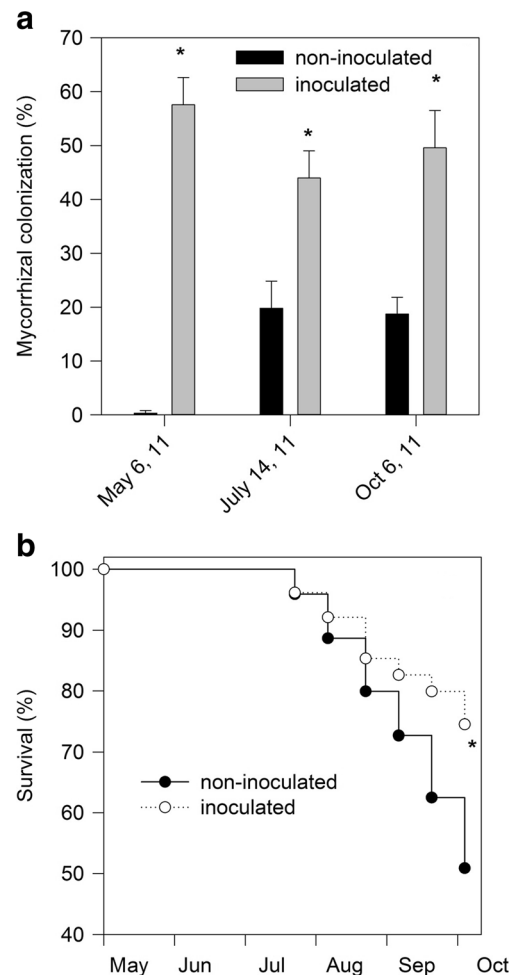


Fig. 1 Total mycorrhizal colonization and survival of sagebrush seedlings during the mesocosm experiment started in the spring of 2011. **a** Mycorrhizal colonization at the time of transplanting (May 6) and 2 and 5 months afterwards. Each bar represents the mean ($\pm$ SE) of at least five seedlings. For a particular date, bars marked by an asterisk are significantly higher than the non-inoculated seedlings ($p < 0.05$) based on an independent t test. **b** Percent of surviving seedlings over the course of the experiment; the asterisk indicates a significant difference ($p < 0.05$) based on a log-rank test

inoculated ones (Fig. 1a, $p = 0.009$). Similarly, arbuscular colonization was lower in non-inoculated than inoculated seedlings, 2.99 (± 0.78) and 10.2 (± 3.95) % ($p = 0.01$), respectively. In October, at the end of the experiment, the difference in total colonization was about 30 % (Fig. 1a, $p = 0.003$). In addition, arbuscular colonization was lower in non-inoculated than inoculated seedlings ($p = 0.03$), which had a median of 0.97 and 8 %, respectively.

Independent of treatment, no mortality occurred until mid-July; 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 (Fig. 1b, $p = 0.011$). By the beginning of September, differences in survival among treatments became apparent. To determine if these differences were related to differences

in the water potential of the soil occupied by roots, predawn water potentials were measured. At this time, no differences in water potential were detected between non-inoculated and inoculated seedlings, which had average values of $-2.76 (\pm 0.49)$ and $-3.08 (\pm 0.55)$ MPa, respectively ($p=0.32$).

The values of colonization of individual seedlings showed a positive (Pearson $r=0.60$) and significant ($p=0.047$) linear correlation with the $\delta^{13}\text{C}$ values of each seedling. This result suggests that increases in AMF colonization were associated with increases in intrinsic WUE of the seedlings. However, the average $\delta^{13}\text{C}$ value of non-inoculated seedlings, while lower on average, was not statistically different from that of inoculated seedlings, $-27.2 (\pm 0.5)$ and $-26.2 (\pm 0.2)$ (‰), respectively ($p=0.35$).

Difference in WUE were neither detected by gas exchange measurements ($p=0.7645$). Furthermore, photosynthetic rates and stomatal conductance did not differ between treatments with p values of about 0.15 (see Supplementary documents, Table 1). As measured by dry biomass, inoculation did not have an effect on seedling growth ($p=0.2$); the dry weight of non-inoculated and inoculated seedlings was $2.06 (\pm 0.66)$ and $1.45 (\pm 0.71)$ g, respectively. Similarly, no significant correlation was observed between the percent colonization of individual seedlings and their corresponding biomass ($p=0.30$). At the time the plants were harvested, the deepest roots had not yet reached the bottom of the pots and no differences were detected in the shoot to root ratio ($p=0.38$), which was $1.58 (\pm 0.15)$ for non-inoculated seedlings and $1.45 (\pm 0.71)$ for inoculated ones.

Fall transplanting mesocosm experiment

During the experiment, average monthly temperatures were within 2°C from those estimated based on a 20-year period (see Supplementary documents, Fig. 2). Precipitation differed more; the fall received 30 mm less precipitation than the average, while the winter and early spring received about 32 mm above normal (see Supplementary documents, Fig. 2). In contrast, late spring and early summer were drier than normal; precipitation measured 30 mm during this period of 2012 compared to the 20-year average of 61 mm.

The effect of inoculation on AMF colonization was similar to that observed in the spring transplanting experiment. At the time of transplanting, AMF colonization of non-inoculated seedlings was negligible, while that of inoculated seedlings was $69.06 (\pm 0.64)\%$ ($p<0.001$). Differences were also noted for arbuscular colonization, with average values of 0.0 and $18.1 (\pm 11.3)\%$ for non-inoculated and inoculated seedlings, respectively ($p=0.04$). Seven and a half months after transplanting, non-inoculated seedlings had lower levels of total AMF colonization than inoculated ones, $23.1 (\pm 5.6)$ and $43.8 (\pm 3.4)\%$, respectively

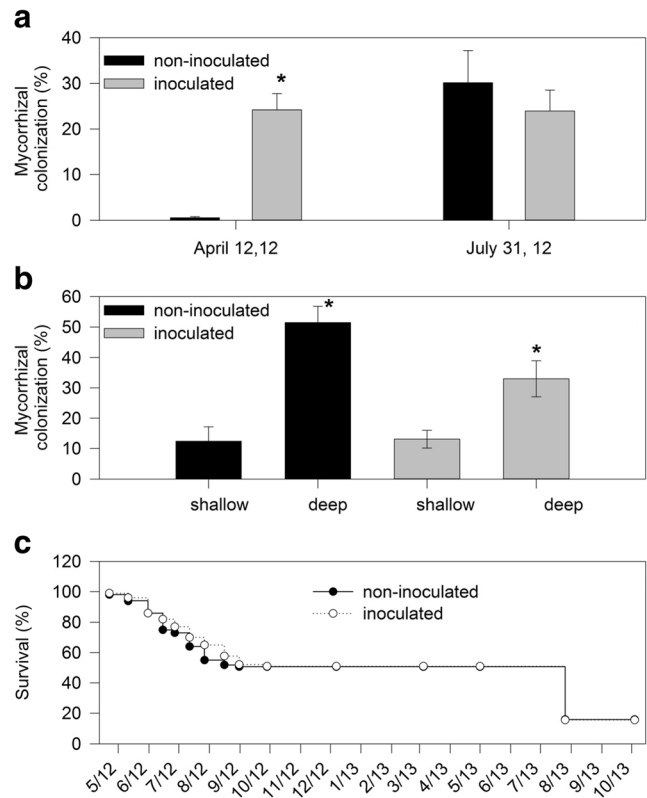


Fig. 2 Total mycorrhizal colonization and survival of sagebrush seedlings transplanted to the Bigfoot site on April 2012. For colonization, each bar represents the mean ($\pm$ SE) of six seedlings. **a** Total colonization per treatment without regard to root depth, bars marked by an asterisk are significantly different from the non-inoculated seedlings ($p<0.05$). **b** Total colonization per treatment in shallow and deep roots collected on July 31, 2012; 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** Percentage of live seedlings over the course of the experiment. Based on a log-rank test, survival was similar among treatments. Symbols in **c** indicate dates when survival was measured

($p=0.007$). Similarly, arbuscular colonization was lower in non-inoculated than inoculated seedlings with median values of 3.15 and 10 %, respectively ($p=0.028$).

Even though inoculation increased AMF colonization, it did not affect the other parameters measured. No seedling mortality was observed for either treatment until June 2012, and at this time, the experiment was terminated because pot size limited root growth. No differences were detected between non-inoculated and inoculated seedling on total dry weight ($p=0.75$), the shoot to root ratio ($p=0.93$) and $\delta^{13}\text{C}$ ($p=0.65$). The total dry weight, shoot to root ratio, and leaf $\delta^{13}\text{C}$ of non-inoculated seedlings were $3.29 (\pm 0.48)$ g, $3.16 (\pm 0.28)$, and $-28.64 (\pm 0.35)$ ‰, respectively. Similarly, the total dry weight, shoot to root ratio, and leaf $\delta^{13}\text{C}$ of inoculated seedlings were $3.54 (\pm 0.60)$ g, $3.12 (\pm 0.38)$, and $-28.92 (\pm 0.51)$ ‰, respectively.

Field experiments

During the spring and fall of 2012, and the spring of 2013, the seedlings experienced temperatures that were similar to the average for the area (see Supplementary documents, Fig. 3a). In contrast, the summers of 2012 and 2013 were about 2 °C warmer than average, while January of 2013 was one of the coldest on record with temperatures 10 °C below average (National Oceanic and Atmospheric Administration, National Weather Service Online Weather Data, <http://www.noaa.gov/>, accessed January 12, 2016). During the experiment, precipitation was lower than normal (see Supplementary documents, Fig. 3b). Cumulative precipitation between April 2012 and September 2013 was 257 mm, which represents 66 % of the average expected cumulative precipitation.

Spring transplanting

At the time of transplanting, total colonization of non-inoculated seedlings was lower than inoculated ones (Fig. 2a, $p=0.01$). Similarly, arbuscular colonization was lower in non-inoculated than inoculated seedlings ($p=0.006$) with median values of 0 and 7.46 %, respectively. Three and a half

months after transplanting, the seedlings had developed a deep tap root, often up to 1 m in depth. We 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). A two-ANOVA of these data indicated no significant interaction between inoculation treatment and root depth ($p=0.08$) and no significant effect of inoculation on total AMF colonization ($p=0.23$, Fig. 2a). In contrast, differences in colonization were evident between shallow and deep roots; total AMF colonization in the shallow roots was lower than in the deep roots (Fig. 2b). After combining results from non-inoculated and inoculated seedlings, the total AMF colonization of shallow and deep roots was 12.7 (± 2.8) and 41.4 (± 4.8) %, respectively ($p<0.001$). 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 ($p=0.02$).

At the time the seedling were sampled for analysis of AMF colonization, no differences in shoot biomass were observed between non-inoculated and inoculated seedlings, which had values of 0.092 (± 0.036) and 0.088 (± 0.034) g, respectively ($p=0.94$). In addition, analysis of foliar $\delta^{13}\text{C}$ revealed no differences between treatments. Non-inoculated and inoculated seedlings had values of -27.51 (± 0.27) and -27.06 (± 0.50) ‰, respectively ($p=0.78$). Furthermore, the correlation between AMF colonization of individual seedlings and their corresponding $\delta^{13}\text{C}$ values, while positive (Pearson $r=0.22$), was not significant ($p=0.49$).

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. 2c). By the beginning of October, the percent survival was 50 % for both treatments. No mortality occurred during the fall, winter, and early spring. However, considerable mortality occurred during late spring and summer of 2013. As a result of this mortality, survival by October 2013 was reduced to about 20 % for both treatments.

Fall transplanting

At the time of transplanting, total AMF colonization of non-inoculated seedlings was lower than that of inoculated ones (Fig. 3a, $p=0.02$). Similarly, arbuscular colonization was lower in non-inoculated than inoculated seedlings ($p=0.01$) with average values of 0 and 6.1 (± 2.4) %, respectively. About 8 months after transplanting, 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 AMF colonization revealed differences between inoculation treatments. Total colonization of

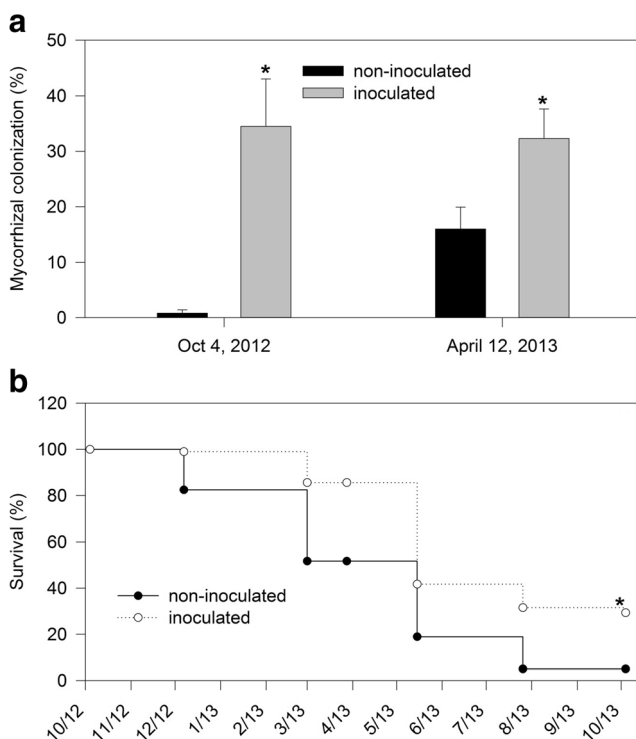


Fig. 3 Total mycorrhizal colonization (**a**) and survival (**b**) of sagebrush seedlings transplanted to the Bigfoot Butte site on October 4, 2012. In **a**, each bar represents the mean ($\pm$ SE) of six seedlings; bars marked by an asterisk are significantly different from the non-inoculated seedlings ($p<0.05$). **b** Percentage of live seedlings over the course of the experiment; based on a log-rank test, survival was higher in the inoculated seedlings. Symbols in **b** indicate dates when survival was measured

inoculated seedlings was twice that of non-inoculated seedlings (Fig. 3a, $p=0.03$). Differences were also observed in arbuscular colonization that had a median of 0.5 and 5.0 % for non-inoculated and inoculated seedlings, respectively ($p=0.007$).

Samples collected in May 2013 showed no significant difference in shoot biomass between non-inoculated and inoculated seedlings, which had values of 0.027 (± 0.006) and 0.048 (± 0.009) g, respectively ($p=0.14$). In addition, foliar $\delta^{13}\text{C}$ values were similar ($p=0.86$), -28.40 (± 0.22) and -28.22 (± 0.32) ‰ for non-inoculated and inoculated seedlings, respectively.

Seedlings transplanted in October 2012 experienced mortality through the fall and winter (Fig. 3a). At the beginning of spring, survival was about 50, and 85 % for the non-inoculated and inoculated treatments, respectively ($p<0.001$). Mortality continued to increase during the spring and summer. By the beginning of fall, 1 year after transplanting, the non-inoculated seedlings had a survival of 4 %, while the inoculated seedlings had a survival of 31 % ($p<0.001$).

Meta-analyses

Across the four studies, AMF inoculation increased colonization after transplanting by an average of 84 % (Fig. 4a, $p=0.0042$, C.I. 21–180 %). Total heterogeneity, Q_T , was

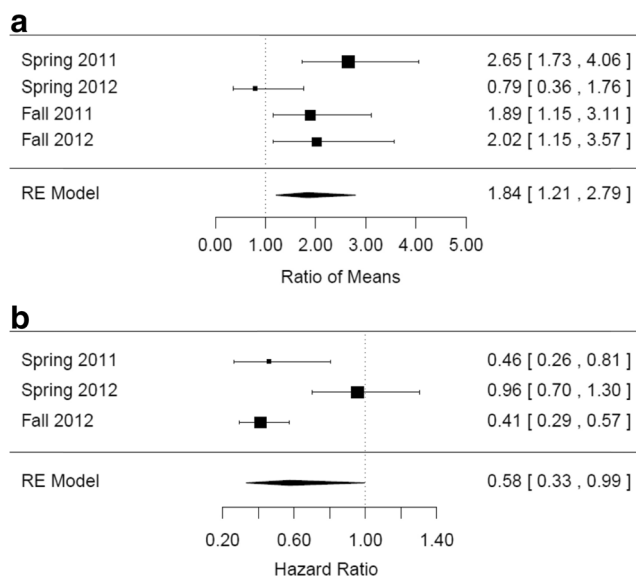


Fig. 4 Weighted and summary responses of sagebrush seedlings to inoculation with arbuscular mycorrhizae estimated by meta-analysis. **a** Mycorrhizal colonization response ratio between inoculated and non-inoculated seedlings and 95 % confidence intervals (CI); a response ratio >1 indicates that inoculation increased colonization after transplanting. **b** Hazard ratio between inoculated and non-inoculated seedlings and 95 % CI; a hazard ratio <1 indicates that inoculation reduced the risk of seedling mortality. Summary responses were calculated by restricted maximum-likelihood estimation (RE model)

6.83 ($p=0.0774$) and the percentage of variation that was due to heterogeneity among studies rather than chance, I^2 , was 56 %. This percent heterogeneity is considered moderate (Higgins et al. 2003) and was entirely attributed to the spring 2012 field experiment. For the experiments in which seedling mortality occurred, inoculation reduced the average risk of mortality by 42 % (Fig. 4b, $p=0.047$, C.I. 1–67 %). However, the heterogeneity was high with a Q_T value of 14.4 ($p=0.001$) and an I^2 of 83 %. Like in the meta-analysis of colonization, all the heterogeneity were attributed to the spring 2012 field experiment. In contrast to the meta-analyses of colonization and survival, the meta-analysis for $\delta^{13}\text{C}$ did not show a significant effect of inoculation on this parameter (data not shown, $p=0.38$). Heterogeneity in this analysis was low with a Q value of 3.62 ($p=0.31$) and I^2 value of 14.7 %.

Discussion

Can the background levels of AMF colonization be increased by inoculation with native AMF?

Based on the results of this study, the AMF propagule density present in the two studied soils was a limiting factor for AMF colonization of Wyoming big sagebrush seedlings. Several months after transplanting, AMF colonization in non-inoculated seedlings was about half of that observed in inoculated ones. This occurred in three of the four experiments, the exception being the spring field experiment. Positive effects of inoculation with native AMF on colonization after transplanting have been observed in other semiarid habitats (Weinbaum et al. 1996; Navarro Garcia et al. 2011). For example, 1 year after transplanting, seedlings of the tree *Cupressus atlantica* inoculated with AMF native to an area in Morocco had 65 % colonization; in contrast, non-inoculated seedlings had only 25 % AMF colonization (Ouahmane et al. 2007). Similarly, in an arid region in Spain, inoculated *Anthyllis cytisoides* seedlings had, 10 months after transplanting, 20 % higher AMF colonization than non-inoculated seedlings (Requena et al. 2001).

For big sagebrush, AMF colonization has been reported to be lower during mid to late summer than at other times of the year (Trent et al. 1994). However, we did not observe such a trend. For the mesocosm experiments, pots were above ground and presumably exposed to larger seasonal fluctuations in at least temperature than if they would have been buried. Nevertheless, in both the mesocosm and field experiments, the colonization measured at different sampling times was similar. The reasons for the discrepancies with other studies are not clear, but they may be attributed to differences in the depth at which the samples were collected. We collected roots to depths of up to 75 and 100 cm for the mesocosm and

field experiments, respectively. In contrast, Trent et al. (1994) collected samples from only the upper 20 cm of the soil. Sampling a larger fraction of the soil profile may have prevented us from detecting differences in AMF colonization in the upper soil layer, which experiences greater fluctuations in moisture and temperature.

In contrast to the other experiments, in the field experiment started in the spring of 2012 inoculation did not increase AMF colonization after transplanting. A similar phenomenon has been observed in Wyoming big sagebrush seedlings transplanted to a disturbed soil (Stahl et al. 1988). For the spring 2012 experiment, the lack of an effect of inoculation on colonization after transplanting may be partly attributed to low initial colonization. In this experiment, inoculation was conducted using Jiffy pellets. Despite our preliminary observations, this approach was not as consistent in yielding high colonization as the use of cone-tainers filled with pot cultures. However, low initial colonization does not appear to entirely account for the subsequent lack of differences between non-inoculated and inoculated seedlings. In the fall 2012 field experiment, the initial level of colonization in inoculated seedlings, while on average higher, was not significantly different from that of seedlings transplanted in the spring. Notwithstanding these similarities, the experiment started in the fall of 2012 showed a positive effect of inoculation on AMF colonization after transplanting.

In addition to low initial colonization, insufficient soil moisture during the spring of 2012 may have negatively affected the ability of the inoculum to spread and colonize the root system (Trent et al. 1994). Clearly, in the summer of 2012, soil moisture appeared to have influenced the distribution of AMF along the root system. At this time, the soil was very dry in the upper 20 cm, but it was moist below 50 cm. This corresponded with differences in colonization that were more than threefold higher in deep, than shallow roots. AMF typically occupy regions in the upper 50 cm of the soil profile, where nutrients and root length density are higher (Jakobsen and Nielsen 1983; Al-Agely and Reeves 1995). Furthermore, various studies have shown that colonization decreases with soil depth (Rillig and Field 2003; Oehl et al. 2005; Yang et al. 2010). However, based on our results, the distribution of soil moisture appears to change this pattern. This may be attributed to preferential growth in areas of the root system in deep and moist layers of the soil. Under these circumstances, AMF colonization would be higher in deep roots because colonization occurs in actively growing portions of the root (Allen 2001).

Are increases in AMF colonization associated with increases in seedling survival?

Our results indicate that increases in AMF colonization can increase survival of 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, fall 2011 mesocosm and spring 2012 field experiments, we did not see an increase in survival. However, in the fall 2011 mesocosm experiment, no mortality occurred during the experiment, while in the spring 2012 field experiment, inoculation did not increase AMF colonization after transplanting.

For the spring 2011 mesocosm experiment, the low values of pre-dawn water potential and soil moisture measured during late summer indicate that a major cause of seedling mortality was summer drought. Under this scenario, the effect of increased AMF colonization on improving survival appears to reflect an increase in drought tolerance. Such effect is consistent with reports in other plant species, which showed AMF-induced improvements in seedling survival in arid environments or in response to artificially imposed drought (Requena et al. 2001; Ouahmane et al. 2007; Abbaspour et al. 2012).

As evaluated by gas exchange measurements and $\delta^{13}\text{C}$ values, the effect of inoculation on increasing survival was not associated with significant differences in WUE between non-inoculated and inoculated seedlings. However, in the spring 2011 mesocosm experiment, the AMF colonization of individual seedlings was positively correlated with their corresponding $\delta^{13}\text{C}$ values. Given that inoculation increased colonization and that colonization was positively correlated with $\delta^{13}\text{C}$ values, it seems likely that, in the spring 2011 experiment, inoculation increased $\delta^{13}\text{C}$ but the sampling effort was not sufficient to detect a difference in this parameter between inoculation treatments. Consequently, further studies are needed to determine whether AMF-induced increases in sagebrush survival during the summer were mediated by changes in WUE.

In the spring 2011 mesocosm experiment, AMF inoculation improved survival, but this did not appear to be related to differences in growth or the ability to explore areas of higher soil moisture. The benefit of AMF to plants has often been measured as a promotion of plant growth. However, Smith et al. (2010) have argued that, in water-limited environments, lack of growth promotion or small growth depressions associated with carbon loss to AMF may be beneficial. Reduced plant size could help to conserve water, while at the same time the AMF hyphae may increase the efficiency of roots to take nutrients and water from a drying soil (Smith et al. 2010). These phenomena could have accounted for the observed AMF-induced increase in survival without promotion of growth. Also, pre-dawn water potentials were measured at a time when seedling losses were occurring, but the values for non-inoculated and inoculated seedlings were similar. In Wyoming big sagebrush seedlings, Stahl et al. (1998) showed that mycorrhizal plants survived lower soil water potentials

than non-mycorrhizal plants. Similar results have been reported for *Glycine max* (L.) and *Phaseolus vulgaris* (L.) (Bethlenfalvay et al. 1988; Augé et al. 2003). In *G. max*, wilting occurred at a lower soil water potential in mycorrhizal than non-mycorrhizal plants (Bethlenfalvay et al. 1988), while in *P. vulgaris* the density of AMF hyphae in the soil was negatively correlated with the soil water potential at which the foliage died (Augé et al. 2003). Perhaps, a similar phenomenon occurred in our experiment, where the effects of similarly low water potentials may have been less damaging to plants with higher rates of AMF colonization. This could have been attributed to higher root hydraulic conductivity, improved soil-to-root contact in a drying soil, and/or access to a larger soil volume in inoculated than non-inoculated plants (Augé et al. 2003; Bárcana et al. 2012; Querejeta et al. 2012).

An increase in survival was also observed in the fall 2012 field experiment. In this experiment, an increase in AMF colonization appeared to have increased tolerance of plants to both cold and drought. At the end of winter, survival in the non-inoculated and inoculated treatments was 51 and 85 %, respectively (Fig. 3b, $p < 0.001$). These results contrast with those observed in the fall 2011 mesocosm experiment when no mortality occurred through the fall and winter. A possible reason for the difference between the mesocosm and field experiment was the severity of the winter, which was much colder in 2012–2013 compared to the winter of 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; Zhou et al. 2012). In addition to increasing survival during the fall and winter, AMF inoculation increased survival during the spring and summer. When only the survival that occurred during the spring and summer is considered, survival in the non-inoculated and inoculated seedlings was 9.7 and 34 % ($p = 0.008$), respectively. These results are consistent with the increase in survival observed in the spring 2011 mesocosm experiment.

We can only hypothesize about the mechanisms by which AMF inoculation increased field survival. The apparent AMF effects on increasing cold and drought tolerance may reflect an AMF effect on growth (Hardie and Leyton 1981; Smith and Smith 2011). Even though we did not detect a significant difference in shoot biomass ($p = 0.14$), in the fall 2012 field experiment, inoculated seedlings had, on average, higher shoot biomass than non-inoculated ones. In various species including a species within the *Artemisia* genus (*A. cana* Pursh), the size and age of the seedlings have been positively related to their ability to develop cold hardiness (Hou and Romo 1998; Kozłowski and Pallardy 2002; Lim et al. 2014).

In addition to growth promotion, AMF colonization can induce other changes in plants that may increase cold and drought tolerance. Cold and drought conditions both increase

the levels of reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, and hydroxyl radical (Cruz de Carvalho 2008; Baek and Skinner 2012). While ROS can act as signals that trigger responses to cope with cold and drought, ROS levels must be regulated to avoid extensive cellular damage (Cruz de Carvalho 2008). Various studies have shown AMF-induced increases in enzymatic and non-enzymatic antioxidants and lower levels of ROS in mycorrhizal than non-mycorrhizal plants, including under water and cold stress (Wu et al. 2006; Miller et al. 2010; Pedranzani et al. 2015). Thus, higher sagebrush survival in inoculated seedlings might have been partly attributable to a reduction in ROS.

While the emphasis of this study is on the effect of increased colonization on seedling survival, another factor that can affect plant responses to AMF is the particular AMF taxa present in the roots (Klironomos 2003; Querejeta et al. 2006). Although we used native AMF, they were multiplied in pot cultures using Sudan grass as a host. Both the host species and greenhouse conditions can alter the proportions of different AMF phylotypes from those occurring in the field (Carter et al. 2014). Consequently, the AMF community of inoculated seedlings, while still native, may differ from that of non-inoculated ones. We are presently investigating this notion, but the possibility cannot be discarded that the seedling responses to inoculation partly reflect differences in AMF composition.

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, mortality was only observed when temperatures were mild to warm and precipitation rare, strongly suggesting that the main factor causing seedling losses 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 January of 2013 (Figs. 2 and 3). Presumably, the more 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 grown before again experiencing cold temperatures. Freezing temperatures shortly after germination may cause losses of sagebrush seedlings (Lambrecht et al. 2007). After surviving through the first summer, however, our results suggest that cold is a minor factor limiting sagebrush establishment.

As discussed above, the effectiveness of AMF inoculation on enhancing colonization and seedling survival varied among the different experiments. Notwithstanding this variation, an integration of the results using meta-analysis indicates

that AMF colonization increased average colonization after transplanting and reduced the risk of mortality. Clearly, these analyses were based on a very limited number of experiments, and more research is needed to identify factors that can affect the ability of the AMF inoculum to colonize roots after transplanting and to determine the extent to which inoculation increases seedling survival across different sagebrush steppe habitats. Questions also remain about the mechanisms by which AMF increased survival during dry and cold periods. Based on the positive correlation between AMF colonization and $\delta^{13}\text{C}$ observed in one of the experiments, the role of intrinsic WUE in increasing survival during the summer merits further investigation. In addition, studies on the effects of AMF on hydraulic conductivity and oxidative stress may provide an insight on whether the AMF-induced increase in survival was mediated by such effects.

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