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NATIVE GRASS AND FORB ESTABLISHMENT IN POST-AGRICULTURAL SOIL

A Thesis

Presented to

the Faculty of Natural Sciences and Mathematics

University of Denver

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Courtney D. Hall

June 2013

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ABSTRACT

Restoration of degraded and abandoned agricultural land in arid and semiarid climates is a global problem. The erratic patterns of precipitation these lands experience makes restoration of a plant community difficult. Application of supplemental irrigation and inoculation with arbuscular mycorrhizal fungi (AMF) are two restoration techniques that have been suggested to overcome deficits in natural precipitation. The effects and the interactions of irrigation and seeding date on the ground cover of intended species and unintended exotic species were tested in a post-agricultural restoration experiment in south-central Colorado, USA. The greatest ground cover of intended species and lowest ground cover of unintended species was observed when seeds were sown in May and were irrigated at higher rates. Results suggest that the timing of sowing as well as the amount of irrigation applied are important in arid post-agricultural restoration. The effects of different AMF inoculation and water treatments on plant biomass were also tested in a manipulative greenhouse experiment. Plant biomass was not greater when inoculated with AMF, which suggests that the use of AMF in post-agricultural soil may not be worth the additional costs of implementation.

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TABLE OF CONTENTS

CHAPTER I: INTRODUCTION.....	6
Agricultural abandonment	7
Restoration	9
Arbuscular Mycorrhizal Fungi.....	11
Conclusion	13
CHAPTER 2: NATIVE GRASS AND FORB COMMUNITY ESTABLISHMENT IN POST-AGRICULTURAL SOIL: EFFECTS OF SEEDING DATE AND IRRIGATION	15
Introduction.....	15
Methods.....	18
Study System	18
Site Description.....	19
Site Preparation and Experimental Design	20
Sampling Methods	21
Data Analysis	21
Results.....	21
Intended species	22
Unintended species	23
Nurse Plants	24
Discussion.....	24
CHAPTER 3: EFFECTS OF ARBUSCULAR MYCORRHIZAL FUNGI INOCULATION IN POST-AGRICULTURAL SOIL: APPLICATIONS FOR RESTORATION.....	30
Introduction.....	30
Methods.....	33
Study system and field collection	33
Preparation of Inoculum	34
Greenhouse design	35
Plant harvest and root assessment.....	36
Data Analysis	37
Results.....	37
Root colonization	37
Plant productivity.....	38

Discussion	38
Conclusion	43
REFERENCES	45
APPENDIX A	56
APPENDIX B	60
APPENDIX C	68

CHAPTER I: INTRODUCTION

Dryland systems provide a multitude of ecosystem services to nearly one-third of the global population. They represent over 40% of terrestrial ecosystems (Safriel et al. 2005), and control many global abiotic processes (Schlesinger et al. 1990). Despite their importance, drylands are being degraded at an alarming rate and few remain in the United States that have escaped anthropogenic damage (Bainbridge 2007). Because of their inherently low productivity, diversity, and relatively low conservation value compared to other ecosystems, (Fredrickson et al. 1998, Bainbridge 2007), research of arid and semiarid lands is rare and/or inadequately funded (Cox et al. 2006). Assessments of biodiversity loss goes unchecked (Ayyad 2003) and their low resilience makes these losses critical. Restorations of species-rich drylands are difficult, costly, and result in low economic returns (Allen 1995, Belnap and Sharpe 1995). This calls for cost-effective and replicable restoration protocols to halt and reverse degradation (UN 2012).

Degradation of North American drylands has continued since the early 1600s (Bainbridge 2007). Droughts, concomitant overgrazing, and lack of conservation policy in this region resulted in intense and acute degradation by the 1800s (Fredrickson et al. 1998). Shifts in land-use from grazing to farming of irrigated crops precipitated large-scale abandonment of farmland during periods of drought in the 1950s (Coffin et al. 1996). Agricultural abandonment continues due to increased water use and purchases of water rights by growing urban and exurban populations, all changing natural hydrological

regimes and reducing water availability for farmers. When abandoned, intensive agricultural practices leave behind severely degraded landscapes, many of which are incapable of a natural recovery once abiotic and biotic thresholds have been passed (Cramer et al. 2008).

Restoration research in the semiarid and arid west United States has a long history that stemmed from growing concern for rancher livelihoods and loss of natural resources. Unfortunately, most work until about 40 years ago focused on quickly stabilizing soils with introduced grasses (Call and Roundy 1991, Allen 1995) to increase forage for livestock (Fredrickson et al. 1998). These efforts taught us little about the ecology of native biotic communities, and caused conservation issues of their own (Call and Roundy 1991, Fredrickson et al. 1998). The goals for revegetation in arid and semiarid land in the United States have changed considerably with the passage of legislation affecting land reclamation (Call and Roundy 1991) and concern for biodiversity loss. However, limited ecological research of desert ecosystems still causes much uncertainty for restoration practitioners today (Bainbridge 2007).

Agricultural abandonment

Large-scale abandonment of arable land has provided opportunities for restoration of native species (Kardol and Wardle 2010), but creates additional obstacles for dryland restorations. Degradation caused by intensive cultivation destroys native seedbanks (Török et al. 2011), reduces soil organic carbon (McLauchlan 2006), alters nutrient levels in soils (Burke et al. 1995), and disrupts soil biotic communities (Cramer et al. 2008,

Kardol and Wardle 2010). Agricultural production reduces the small-scale heterogeneity of the soil by distributing nutrients evenly across the landscape, degrading and reducing variation of “resource islands”, features necessary for maintenance of biodiversity in these ecosystems. Native perennial bunchgrasses are adapted to low nutrient and moisture levels in soils and have an advantage over annuals in these conditions (Belnap and Sharpe 1995). However, early colonizers of abandoned agricultural land in the western US, such as *Kochia scoparia*, increase N availability under their canopies, thus increasing their own competitiveness (Belnap and Sharpe 1995) and reducing overall heterogeneity of the soil (Burke et al. 1998). Agricultural abandonment leaves soils barren and unprotected from wind erosion. Strong winds are common in semiarid and arid grasslands and are the driving force behind redistribution and deposition of soil important in the formation of resource islands (Burke et al. 1998). Wind erosion reduces the silt content of the soil (Burke et al. 1995), causing shifts in soil texture that can preclude both natural and assisted establishment of key plant species, because of reduced moisture holding capacity (Lauenroth et al. 1994).

Although agricultural disturbance may be mechanically similar to other abandoned areas in the United States, the recovery patterns of arid and semiarid systems may be different because of unique site characteristics and historic factors (Coffin et al. 1996). Stopping disturbance alone does not always result in ecosystem recovery (Curtin 2002). Centuries can pass before abandoned fields in drylands are able to recover perennial plant communities (Coffin and Lauenroth 1994, McLendon et al. 2012) and legacy effects on soils can take millennia to recover (McLauchlan 2006). Drylands may

never return to their original state (on a human timescale) due to slow and stochastic natural successional processes (Allen 1995, Scheffer et al. 2001) and therefore, will require substantial intervention.

Restoration

Arid and semiarid grasslands are shaped by the low and unpredictable precipitation patterns typical of these areas. Limited water availability affects N availability, C decomposition and storage, and aboveground biomass production (Lauenroth and Bradford 2006). Erratic precipitation creates substantial obstacles for restoration practitioners trying to re-establish native vegetation (Call and Roundy 1991, Grantz et al. 1998, Padgett et al. 2000). If supplemental irrigation is not available and restoration is dependent on natural precipitation regimes, a good year for seeding and plant establishment may only occur once or twice in 15 years (Bleak et al. 1965). Therefore, restoration success varies considerably among years (Grantz et al. 1998, Wilson et al. 2004). Sufficient moisture levels are the most critical aspect for natural seedling establishment in arid and semiarid systems (Padgett et al. 2000, Myers and Harms 2011), yet there is little known about the minimum critical moisture level for intentional revegetation in these areas (Allen 1995).

Temporary irrigation may alleviate water limitations for vegetation establishment. However, there are few studies that directly manipulate irrigation levels in arid and semiarid restoration projects (Table 1A). Those that do supplement moisture have had varying outcomes, making ambiguous any conclusions on irrigation's usefulness (Abella

and Newton 2009). In a coastal shrub restoration in California, irrigation heavily favored a single species, *Artemisia californica*, over five other species, reducing overall diversity of plots (Padgett et al. 2000). Belnap and Sharpe (1995) found that un-irrigated plots of *Stipa* grasses performed as well as irrigated plots. However, cover of exotic *Salsola* spp. was 70% higher when plots were irrigated, in a cold desert restoration in Utah. The closest that we have come to protocols for irrigation in restoration has been from Roundy et al. (2001), who suggested using irrigation to encourage emergence of seedlings before the onset of summer rains. They had considerable success keeping soil moisture levels at field capacity for only 10 days after seeding and recommended using between 200-300mm of supplemental water to accomplish this. Yet, we are unaware of studies that have replicated his design.

Trying to draw conclusions from the literature on restorations using irrigation to establish species-rich communities is difficult. Re-vegetation in drylands has been successful in dry years and unsuccessful in wet years, and suggests that there are other barriers than soil moisture to re-vegetation in these systems (Abella and Newton 2009). Almost two decades have passed since the call for better understanding of moisture management for dryland re-vegetation (Allen 1995). Yet this issue is mainly overlooked, and we as a community are no closer to forming recommendations for land managers. The direct manipulation of water and its effect on different plant species may help elucidate the usefulness of irrigation in desert re-vegetation (Abella and Newton 2009).

Arbuscular Mycorrhizal Fungi

Literature on the link between below-ground and aboveground processes and its control over ecosystem diversity and function has been increasing over the few decades. Once thought of as a passive environment for resource competition of plants, soil is now recognized as an interactive component of a larger network (Kulmatiski et al. 2006) that contributes greatly to plant diversity and function (Van der Heijden et al. 1998b, Collins and Foster 2009). Soil microorganisms have been shown to be important in soil formation (Rillig and Mummey 2006) and biogeochemical processes (Van der Heijden et al. 2008). For instance, in nutrient-poor ecosystems, soil microorganisms provide up to 90% of N and P to plants (Van der Heijden et al. 2008). Despite the substantial research on soil-plant biotic interactions, restoration projects incorporating both components are still scarce (Kardol et al. 2009, Harris 2009, Kardol and Wardle 2010). Plant-soil biotic interactions may make the difference between a successful restoration or a failure (Eviner and Hawkes 2008).

Restoration practitioners have long recognized the importance of the interaction between plants and AM fungi. There have been decades of theoretical evidence from greenhouse microcosms that can be used to inform restoration. However, because of the stochastic environmental factors, site-history factors, and complex 3-way interaction between soils, fungi, and plants, not all restorations using inoculation strategies have been successful (Table 2A). Without active restoration, AM fungi communities must rely

on natural dispersal of spores to colonize sites. This process can be slow, especially in highly disturbed environments (Allen 1989), and early colonizers on semiarid and arid sites, such as *Salsola kali* and *Kochia scoparia* (Renker et al. 2004), tend to be non-mycotrophic.

Restoration studies have suggested that in post-agricultural restorations native soil inoculation of seedlings can accelerate successional processes by benefiting late-seral species and negatively affecting early-seral species (Rowe et al. 2007, Middleton and Bever 2012). Soil inoculation can increase native species cover by increasing their competitiveness over unwanted weeds (Smith et al. 1998). Inoculations with different native whole-soils (as opposed to extracted spores or biotic components) resulted in higher plant biomass and evenness in microcosm grassland experiments in the Netherlands (Carbajo et al. 2011). However, these effects were less pronounced when native whole-soil was applied to arable organic soil, than soil that had had topsoil removed (Carbajo et al. 2011), suggesting soils with high nutrient levels may lessen benefits of inoculation. It has been widely reported that AM fungi may act antagonistically on host-plants when nutrients are not limiting in the soil (Johnson 1993, Johnson et al. 1997, Hoeksema et al. 2010). However, in a greenhouse study using degraded soil from a restoration site in Rocky Mountain National Park., Rowe et al. (2007) demonstrated that different levels of P had little effect on plant responsiveness to AM fungi. They also found that commercial inocula were ineffective at colonizing plant roots of herbaceous montane species, and did not confer benefits to growth compared to an uninoculated control.

Restoration studies integrating AM fungi inoculation have occurred in diverse climates, in sites of varying states of degradation, and have used native whole-soil, native AMF-only, and commercial inoculation sources. It is difficult, therefore, to draw definitive conclusions on what works and what doesn't, and why this might be. Because of our narrow focus on plant growth or colonization, we may be unintentionally limiting our understanding of the importance of AM-fungi mediated restorations. For example, in a re-vegetation experiment in Wyoming, inoculation did not result in higher relative cover of species planted, but inoculated native perennial plants experienced less competition from annuals during periods of drought (Allen and Allen 1986). We also know that AM-fungi are important for a wide range of ecosystem functions, which are often in a degraded state prior to restoration. Requena et al. (2001) showed that inoculation with a native AM fungi mix improved soil aggregation, an important aspect of soil structure and function. Lastly, we need to be more explicit describing site characteristics of our restorations. We know that AM fungi can act antagonistically on plants when nutrient levels are high in the soil. Yet, not all restoration practitioners fully describe the soil characteristics of their own sites (Table 2). Quantitative data are required more than subjective descriptions. Without this information, our ability to draw conclusions from these experiments is hindered, and can be inaccurate.

Conclusion

Sweeping generalities of techniques that worked or that did not work are sometimes based on just a few studies. Unfortunately, it seems that the difference

between success and failure of restoration projects is more dependent on site-specific characteristics than regional parameters of soils and plant communities (Eviner and Hawkes 2008). This may be a consequence of a young and developing science. Restoration of degraded lands is not dependent on ecological science alone. It requires collaboration among land managers, policy makers and scientific and engineering disciplines (Havstad et al. 2007). Restoration scientists should seek out collaborations with practitioners in the field (Menz et al. 2013) to expand the breadth of understanding of different ecosystems and techniques. Results from these studies should be published whether they worked, or did not, and especially when the experiments were replicated. There are no 'quick fixes' in restoration of semiarid and arid lands.

CHAPTER 2: NATIVE GRASS AND FORB COMMUNITY ESTABLISHMENT IN POST-AGRICULTURAL SOIL: EFFECTS OF SEEDING DATE AND IRRIGATION

Introduction

Dryland degradation caused by unsustainable agricultural production is a problem faced on all major continents (Safriel et al. 2005). Economic incentives and technological advances in farming have accelerated the conversion of natural systems leading to substantial losses in biodiversity (Dobson et al. 1997), which is often unaccounted for in these neglected ecosystems (Fredrickson et al. 1998, Ayyad 2003). Over-irrigation and unsustainable withdrawal of groundwater (Richter et al. 2002) has reduced primary production, decreased water-related ecosystem services, and caused soil-related problems such as increased salinity and erosion (Grantz et al. 1998, Safriel et al. 2005). Cost-effective and replicable restoration protocols to halt and reverse dryland degradation are needed.

Former and continued biotic and abiotic agricultural stresses can drastically reduce an ecosystem's ability to return to its natural state when abandoned (Kulmatiski 2006, Cramer et al. 2008). Intensive cultivation can preclude later natural recruitment by destroying the native seed bank and altering nutrient levels in the soil, favoring persistent weeds (Kulmatiski 2006, Cramer et al. 2008, Kardol and Wardle 2010, Török et al.

2011). Secondary succession is hindered further in arid environments (Call and Roundy 1991) due to both the unpredictable timing of abundant precipitation (Grantz et al. 1998) and intense winds (Burke et al. 1995). Over a century can pass before old fields are able to recover a persistent perennial community (McLendon et al. 2012). If undesirable species establish, later active restoration may prove too costly and impracticable (Seastedt et al. 2008). With land abandonment increasing rapidly, it is important to investigate abiotic and biotic constraints to plant assembly to determine whether restoration efforts are sensible (Cramer et al. 2008).

Restoration of a native, species-rich community is difficult in arid and semiarid systems (Allen 1995, Padgett et al. 2000, Banerjee et al. 2006, Abella and Newton 2009) and successes in post-agricultural grassland restorations have been limited (Richter et al. 2002, Banerjee et al. 2006). Regardless of how well a restoration is planned, the low and unpredictable nature of precipitation can preclude germination and limit the establishment of a persistent perennial community (Call and Roundy 1991, Coffin and Lauenroth 1994, Grantz et al. 1998, Padgett et al. 2000). If dependent on natural precipitation regimes, a favorable year for restoration in an arid system may occur only once or twice in 15 years (Bleak et al. 1965), which makes replication of successful re-vegetation between years unlikely (Grantz et al. 1998, Wilson et al. 2004).

Temporary irrigation may be a technique to overcome erratic moisture patterns and associated limitations in plant establishment. However, there are few restoration studies that have directly manipulated irrigation and those that did had highly variable results (Abella and Newton 2009). Some have found that irrigation treatments did not

improve restoration success and that irrigation was detrimental to several native species (Padgett et al. 2000). Drought-tolerant species may be overwhelmed when restoration techniques include irrigation, resulting in domination of communities by moisture-tolerant species and failure when supplemental water stops (Allen 1995). Additional water may also increase competitive advantages of exotic annuals. Belnap and Sharpe (1995) demonstrated that irrigation did not increase establishment of native *Stipa* grasses and resulted in 70 percent higher biomass of exotic *Salsola* spp. Other studies, however, have supported that irrigation improves the establishment of herbaceous species on abandoned farmland in the Sonoran Desert (Roundy et al. 2001) and significantly increases shrub survival and growth in dry years on barren farmland in California (Yamashita and Manning 1995). The direct manipulation of water and its effect on different plant species may help elucidate the usefulness of irrigation in re-vegetation (Abella and Newton 2009).

The seemingly simple choice of when to plant or sow seeds is a decision that is faced by all restoration practitioners regardless of climate, and may have dramatic consequences on later community composition (Körner et al. 2008, Martin and Wilsey 2012). Martin et al (2012) found that spring planting resulted in higher abundance and diversity of natives, and had fewer exotics than summer planting. Exposure of seeds to moisture and temperature cues via dormant season planting, on the other hand, may allow for earlier germination in the spring (Larson et al. 2011). Earlier spring germination can influence community assembly (Körner et al. 2008) and may be important for competition with annual weeds (Verdu and Traveset 2005). However, dormant season

planting may also leave seeds vulnerable to attack by parasitic or detrimental fungi and herbivores.

Experimental restoration studies provide excellent opportunities to explore the factors that affect plant assemblages (Collinge and Ray 2009) and these types of studies are lacking in arid post-agricultural systems. In 2008, the Natural Resources Conservation Service (NRCS) and Colorado State University (CSU) Extension began testing restoration techniques to inform a 16.2 thousand hectare Conservation Reserve Enhancement Program (CREP) restoration. The purpose of this re-vegetation effort was to determine whether the installation of a native herbaceous cover in arid post-agricultural fields can be accomplished economically and with minimal initial irrigation. Here, we report the results of a four-year revegetation experiment examining the effects of biotic and abiotic manipulations on the restoration of an herbaceous community. In a full factorial design we manipulated amount of supplemental water, seeded species, and seeding date. We evaluated these effects and their interactions on the ground cover of planted and non-planted exotic species.

Methods

Study System

The San Luis Valley is an extensive, flat valley in south-central Colorado covering an area of 8300 km². It is located between the Sangre de Cristo and the San Juan Mountains, situated at 2350 m elevation. The valley is subject to very high winds from the west and north, and low precipitation averaging only about 180mm/yr. with most

precipitation occurring as monsoon rains in July through September (Emery 1979, Cooper et al. 2006, Kray et al. 2012). Although this is a true desert, the valley sits over considerable ground water stored in two major aquifers. Intensive irrigation is essential for crops (mostly potatoes and barley) in this area, and aquifer draw-down has severely decreased ground water levels, movement and discharge (Emery 1979, Bexfield and Anderholm 2010). Dwindling water resources has decreased productivity and exacerbated the severity of wind-blown soil movement soliciting the need of widespread retirement of cropland and restoration to a viable plant community.

Site Description

The experimental site was located near Hooper, Colorado (37°43'41"N, 105°58'25"W) on a 3-ha private field corner owned by Zapata Seed. The site had been previously irrigated for grain and potato production over 30 years ago. A single crop of potato minitubers was planted and harvested over 10 years ago. Since then, the site has been in annual weed cover. The soil at this site is predominantly a moderately-well drained McGinty sandy loam (NRCS 2012). The site is surrounded by actively cultivated fields with nearby undisturbed vegetation consisting of greasewood (*Sarcobatus vermiculatus*) and rabbitbrush (*Ericameria nauseosa*), although the dominance of these shrubs may be partially a result of a disturbed hydrological system (Cooper et al. 2006).

Site Preparation and Experimental Design

In the fall of 2008, a winter wheat cover crop was planted at the test site. In 2009, the site was irrigated several times with a total of 33mm of water and was divided into 4 equal-sized quadrants. Each quadrant was seeded on different dates (May 2009, July 2009, November 2010 and April 2010) for a “seeding date” treatment. Quadrants were divided in half, with each half receiving a different irrigation treatment. Under CREP stipulations, restoration of agricultural land in the San Luis Valley will be limited to 18 inches (457 mm) of irrigation over three years. Our irrigation for this study reflects this CREP specification. Irrigation treatment 1 (IT1) aimed to irrigate within the management allowable depletion, or between permanent wilting point and field capacity of the soil. For irrigation treatment 2 (IT2), supplemental water was used to keep soil moisture just above the permanent wilting point. Amounts of irrigation are provided in table 2. Prior to seeding, glyphosphate was applied to kill the cover crop and annual weeds. Seeds were drilled directly into the dead cover with a plot-drill set to a 1.27cm seeding depth. Four 4x9-m replicate plots of single species of native grasses, mixed forbs, and non-native grasses were assigned to each of the irrigation and seeding date treatments (see Table 1B for seeded species). Replicated zones of mixed native grasses and forbs were planted around and between blocks of plots (Figure 1B). A buffer zone of mixed grasses was sown around the plots to reduce colonization of species between plots. The field was irrigated using an existing mini-pivot sprinkler from 2009 through 2011.

Sampling Methods

In June 2011, the last year of irrigation treatment, plant cover was measured using the line intercept method. Two diagonal transects measuring 8m were placed across all grass and forb plots and 16m transects were laid out in mixed species areas. Non-planted exotic species were not included in this ground cover estimate. After irrigation had stopped, plant cover was measured in August 2012. Plant cover was estimated by placing a 0.25m² quadrat at 4 random intervals along a diagonal transect in all plots. Soils were sampled in November 2012 from May and July seeding date treatment plots. Samples were collected from five, randomly selected points, thoroughly homogenized and were sent to the Colorado State University Soils Laboratory for analysis (Table 3B).

Data Analysis

The effects of irrigation, seeding date, species treatments, and their effects on relative plant cover were tested using analysis of variance (ANOVA). Relative cover data was square-root transformed to meet statistical assumptions. When significant results were found, means were compared by post-hoc Tukey's HSD tests. All statistical analyses were performed with JMP Pro 10.0.0 (SAS Institute Inc., Cary, NC).

Results

In 2009, a bi-modal precipitation pattern that diverged from historical patterns was observed in the San Luis Valley (Figure 2B) (Western Regional Climate Center 2013). Around two-thirds of annual precipitation typically occurs as monsoon rains from

July-September (Cooper et al. 2006). However, during the first year of seeding, a large amount of precipitation fell in April, May, September and October. Overall precipitation was slightly above average in 2009. During the second year of seeding trials, there was substantial drought in the months following the April seeding as well as during the month of November seeding. Irrigation in IT1 plots added an additional 193 – 437mm of water during the first year. Irrigation in IT2 plots added 173 – 302 mm of extra water during the first year of establishment. Details of irrigation are provided in Table 2B.

Intended species

Species seeded in April and November 2010 failed to establish and those plots were dominated by annual weed cover from *Kochia scoparia* and *Salsola kali*. Results from these treatments have been excluded from the analysis. When sampled in June 2011, May planting date ($F_{1,171}=27.9$, $p<0.0001$) and IT1 irrigation treatment ($F_{1,171}=19.3$, $p<0.0001$) had significant positive effects on overall plant cover after three years of irrigation. This did not change when sampled the following year in August 2012, a year after irrigation had stopped (Figure 3B).

Not all sown species performed equally, and some failed to germinate. *Stipa hymenoides* and *E. elymoides* had limited emergence in all plots regardless of the irrigation or planting date treatments. Although *S. hymenoides* and *S. cryptandrus* fared slightly better in the dormant season planting, their overall establishment was poor (results not shown). There was a significant interaction between planting month and species planted ($F_{10,131}=3.4$, $p<0.01$). Almost all species performed better when seeded in

May (Figure 4B). *Elymus elymoides*, *S. cryptandrus*, and the mixed species treatment plots were exceptions, with slightly higher cover in plots when seeded in July. *Elymus elymoides* responded poorly to all treatments and had less than 1% relative cover in any treatment. *Sporobolus cryptandrus*, which had nearly no plants establish after 4 years, performed better in July, although cover was still less than 5%. Post-hoc tests, however, revealed that differences within species treatments for planting month were only significant for *B. gracilis* whose cover was higher when planted in May (Tukey's HSD $\alpha < 0.05$). When planted in July, cover of *B. gracilis* was less than 1%.

Unintended species

There was a significant interaction effect between irrigation and planting date treatments on weed cover ($F_{1,171} = 16.6$, $p < 0.0001$). In May, plots under IT1 irrigation had lower weed cover, however in July, plots under IT2 had lower weed cover (Figure 5B). May planting with IT1 irrigation had significantly less weed cover than all other treatment combinations (Tukey's HSD $p < 0.05$). There was a significant interaction effect of irrigation treatments and species planted on weed cover ($F_{10,131} = 2.1$, $p < 0.05$). Most plots had less weed cover under wet conditions (Figure 6B). However, mixed species, *E. elymoides*, and *E. lanceolatus* treatment plots had lower weed cover under dry (IT2) conditions. The reduction in weed cover compared to IT1 plots within these 3 species was small and not significant (Tukey's HSD $\alpha < 0.05$). All differences in irrigation within species treatments were found insignificant except for *P. smithii*. Under IT2 irrigation

treatment, weed cover in *P. smithii* plots was 21.1%. T1 irrigation reduced weed cover to just 4.5% in *P. smithii* plots.

Nurse Plants

We had not intended to use nurse plants to aid grass establishment as a part of our restoration strategy. In the field, however, we observed grasses growing under *Artemisia ludoviciana* that had self-seeded outside of the forb treatment plots. We tested the effects of *A. ludoviciana* on the establishment of grasses and its ability to exclude exotic weeds. In conditions that were optimal for grass growth (May, IT1), having sage in a sampling plot was negatively associated with having grass present (pearson $\chi^2=10.4$, $p<0.001$). However, in conditions that were not optimal for grass growth (July, IT2), we were more likely to find grasses when sage was present in a sampling plot, however this was not significant at $\alpha < 0.05$ (pearson $\chi^2=3.03$, $p=0.08$). For *B. gracilis*, one of the poorest performing grasses in this study but a dominant species in nearby native grasslands, cover increased when sage was present in the sampling plot. Having sage in a sampling plot also reduced the incidence of weeds under all conditions (pearson $\chi^2=68.3$, $p<0.0001$) and reduced weed cover under all treatments ($F_{1,173}=12.8$, $p < 0.001$).

Discussion

The results from our study show that while irrigation was important in some months, additional water did not always result in successful native plant establishment. The dormant season and early spring season seeding plots were dominated by annual exotics,

with minimal emergence of seeded natives regardless of the irrigation treatment. Irrigation was beneficial to native plants in both the May and the July seeding treatments. However, May seeding plots had greater native cover than any other seeding date treatment, and when irrigated to field capacity, had significantly lower weeds. We also observed that plots seeded with mixed grasses and forbs resulted in higher ground cover and a reduction in annual exotics compared to plots that were sown with only a single species or functional type (either grasses or forbs). These findings suggest that water is not the only barrier to native plant establishment in arid systems (Abella et al. 2009), and that attention should be paid to biotic factors such as plant interactions and the timing of sowing.

Soil moisture is an important abiotic filter that limits seedling recruitment in herbaceous communities (Myers and Harms 2011) and the availability of soil moisture is the most important aspect for seedling survival in arid and semiarid systems (Padgett et al. 2000). Nevertheless, there is little information on the minimum moisture threshold for native species establishment in arid systems (Allen 1995). We found that plots irrigated to field capacity had higher ground cover of native species than plots where irrigation was limited. Although we seeded species that are adapted to mesic or xeric systems, some species still require high levels of initial moisture to establish (MacDougall et al. 2008). Our study agrees with other studies that kept moisture levels high during establishment (Roundy et al. 1997, 2001). Warm season grasses need long periods of moisture in the upper soil surface level to develop adventitious roots (Roundy et al. 1997). Roundy et al. (2001) found that most species benefited from maintaining available soil moisture in the

upper 3cm of the soil. Although some restorations using supplemental water have been less successful, Banerjee et al.(2006) speculated that direct seeding failed in their post-agricultural re-vegetation in Arizona because irrigation was applied insufficiently.

Timing of sowing may also play an important role in restoration, with earlier germination of natives conferring a competitive advantage when the number of and proximity to neighbors are low (Ross and Harper 1972, Zimmermann et al. 2008, Wainwright et al. 2012). While perennial species benefit more from early emergence than annuals (Verdu and Traveset 2005), perennials have more restricted germination cues than annual exotics (Wainwright et al. 2012). Exotic annuals have the ability to germinate under a variety of environmental conditions and/or under small resource pulses. It may be that plots sown with native species in April, July, and November had low cover because the natives could not germinate in these conditions. Germination plasticity of the exotics, however, may have allowed *Kochia scoparia* and *Salsola kali* to germinate under conditions adverse to native species. This would have allowed them to outcompete germinating natives by preemptive capture of moisture provided by irrigation via priority effects, and to persist in most plots. Other studies have demonstrated how priority effects of earlier exotic arrival can create persistent stands of near monocultures (Dickson et al. 2012).

Annual plants are thought to have a competitive advantage over native species when moisture and nutrient levels are high (Belnap and Sharpe 1995). However, our study shows that weed cover is contingent upon seeding date of natives and the amount of irrigation applied. Irrigation in our study may have encouraged plants to germinate earlier

to outcompete weeds (Firn et al. 2010). Under IT1 and when natives were seeded in May, exotic cover was significantly lower than in any other treatment, leading us to believe that there are important biotic and abiotic interactions in community assembly (Zimmermann et al. 2008, Firn et al. 2010). A study by Körner et al. (2008) demonstrated that priority effects can persist. They showed that even a 3-week earlier arrival time of one functional group can significantly change community composition later on. When grasses were sown first, the subsequent community had significantly less forbs than when grasses were sown first and the opposite was true when forbs were sown first (Körner et al. 2008). This may explain the early advantage given to species seeded in May and plots irrigated to field capacity, and why advantages persisted over the length of our four-year sampling period. Martin and Wilsey(2012) also demonstrated lasting effects from earlier plantings. Their spring plantings had higher abundance and diversity 6 years after sowing, whereas other treatments were still dominated by exotics. However, a study by Abbott and Roundy (2003) found that germination in a semiarid grassland responded to the total available soil moisture and not the date of seeding, and they suggested that failure of seedlings was due to seedling desiccation.

Our study was limited in a couple of ways: 1) it was restricted to a small site and 2) there was only one year of sampling after irrigation had been turned off. The San Luis Valley is an actively cultivated area with nearly 260,000 hectares of land in production (Emery 1979). Increasing the geographic breadth of our study would have incurred substantially more costs mostly to compensate farmers for four years of forfeited earnings. Since our site had been previously cultivated and irrigated, it represented areas

targeted for future restoration. Only one sampling year after cessation of irrigation may not adequately demonstrate restoration outcome because effects of drought on community composition are not often observed until the fourth year of drought (Evans et al. 2011). Other studies have shown a reduction in perennial grass cover and increase in cover of annual weeds with frequent summer droughts (Morecroft et al. 2004). Although we expect the composition of this community to change over time, it doesn't detract from insights gleaned from this study; that native grass and forb establishment is possible in this area, and that it requires little financial investment or effort once seeds have been sown. Also, studies performed in other arid, post-agricultural areas have demonstrated that annual weeds common to our site decrease in a relatively short time (Wali 1999), may facilitate grass establishment in harsh environments (Allen 1989), and reduce herbivory on native bunchgrasses (Belnap and Sharpe 1995)

We have demonstrated the importance of plant interactions as they relate to restoration four years after initial establishment. Our study suggests that *A. ludoviciana* may aid as a nurse plant in native grass establishment and later natural recruitment, and to our knowledge, we are the only study to have evaluated both its facilitative effect on native grasses and competitive ability against exotic weeds. It may be possible to increase the seeding rate of a native nurse plant in order to reduce costs of restoration by lowering the amount of supplemental water required for establishment, or to produce self-sustaining communities. Research focusing on competitive plant-plant interactions dominates the ecological literature (Brooker et al. 2008). Our work highlights the need to examine facilitative plant interactions in arid restorations. Our study also encourages

future restoration studies to investigate factors that affect community assembly. Had we ignored seeding date in relation to irrigation, we may have had complete failure or confounding success. An obvious next step for this study would be to test replication on a larger scale, and will be easy to do with the collaboration of land managers involved with a CREP restoration on a landscape scale.

Due to increasing land abandonment and the expansive areas of degraded drylands worldwide (Bot et al. 2000, Lepers et al. 2005, Safriel et al. 2005), it is critical that restoration practitioners test easily replicable techniques. This study was low-cost and required few additional resources that a farmer using center pivot irrigation does not already have. It also provides insights on when to seed and how this affects later community composition. Although it may often seem better to look at the germination requirements of individual species, it is more straightforward to prepare one seed mix and plant at one time (Frischie and Rowe 2012). Our study demonstrates the benefit of using irrigation when sowing has been timed effectively and shows plant-plant interactions important for facilitating successful restorations in arid systems.

CHAPTER 3: EFFECTS OF ARBUSCULAR MYCORRHIZAL FUNGI INOCULATION IN POST-AGRICULTURAL SOIL: APPLICATIONS FOR RESTORATION

Introduction

Accelerated rates of degradation in arid and semiarid agricultural systems demands cost-effective and replicable restoration protocols to improve ecosystem services and function (Hobbs et al. 2006). Arid and semiarid systems create unique obstacles for restoration practitioners. Projects to restore semi-arid and arid lands have had variable success because of harsh climatic conditions such as erratic and unpredictable precipitation. Degradation caused by intensive agriculture further complicates dryland restoration by altering biotic and abiotic conditions of the soil (Cramer et al. 2008). Abandoned fields are left with damaged and barren soil susceptible to wind erosion, as well as altered levels of soil biota, nutrients, and salts.

Restoration scientists have long recognized the importance of arbuscular mycorrhizal (AM) fungi in arid land restorations (Allen 1989). These ‘keystone mutualists’ (O’Neill et al. 1991) form symbiotic relationships with approximately 80% of all land plants and develop extensive mycelial networks belowground exchanging nutrients for photosynthate with host plants (Smith and Read 2008). Their extra-radical

hyphae are important for improving soil aggregation (Requena et al. 2001, Hallett et al. 2008), carbon sequestration (Johnson et al. 2010) water infiltration, and mitigating erosion (Rillig and Mummey 2006, Barrios 2007). AM fungi exhibit strong bottom-up control that contributes significantly to plant diversity (McCain et al. 2011) and function (Van der Heijden et al. 1998b, Rillig 2004, Hausmann and Hawkes 2010), and have been shown to alter the competitive ability of invasive plants (Callaway et al. 2004). Studies have demonstrated the effectiveness of AM fungi to shift plant community composition away from one dominated by ruderals (Busby et al. 2011) or warm-season grasses (McCain et al. 2011) in disturbed sites or in low-diversity restorations. AM fungi have also been shown to increase seedling establishment (Van der Heijden 2004). Therefore, AM fungi may be a critical component for successful restoration of abandoned agriculture (Richter et al. 2002, Kulmatiski et al. 2006, Kardol and Wardle 2010).

Agricultural soil disturbances such as tillage and planting of non-mycorrhizal crop plants may significantly reduce the density and diversity of AM propagules in the soil as well as the functioning of existing AM communities (Johnson and Pfleger 1992, Johnson et al. 1997, Kulmatiski et al. 2006, Middleton and Bever 2012). Early plant colonizers on abandoned agricultural land are mostly non-mycorrhizal, which may preclude or slow reestablishment of AM fungi and the native plants that depend on these obligate symbionts (Allen 1989). The lack of mycorrhizae in the soil is particularly critical in drylands (Marulanda et al. 2007) because of their role in alleviating drought stress

(Davies et al. 1992, Augé 2001) and in exploiting limiting nutrients (Smith and Read 2008, van der Heijden et al. 2008). Efforts to reintroduce native plants in degraded drylands may be hampered by the loss of diversity of microorganisms in the soil (Requena et al. 1996, 2001, Marulanda et al. 2007).

Compared with the extraction and propagation of native AM fungi, commercially available AM fungi may reduce the difficulty and cost associated with large-scale inoculation strategies in restoration. However, commercially produced inocula does not always enhance plant vigor (Schwartz et al. 2006) or improve restoration success (White et al. 2008). Due in part to the complicated interactions that AM fungi have with native plant communities and problematic invaders (Richardson et al. 2000, Callaway et al. 2004), concern for the consequences of introducing foreign inocula in restoration is drawing increased awareness (Schwartz et al. 2006). Whereas commercial AM inocula are generalist fungi selected for their ability to quickly propagate, native fungi may be adapted to the limiting factors of targeted restoration sites. For example, native, presumably drought-tolerant fungi have been shown to be better than non-native fungi of the same species at alleviating drought stress in plants (Marulanda et al. 2007). Therefore, the use of native AM fungi from an arid environment may be better at reducing water stress in plants. The use of native AM fungal inocula has been advised when obtainable; however, availability may be limited in a vastly disturbed agricultural landscape, or may damage soils during extraction. Also, given extensive research on ecotypic matching of AM fungal communities to edaphic conditions (Johnson et al. 2010, Ji et al. 2010), it is

uncertain whether using a native inocula source would be beneficial in a highly altered soil environment.

This study evaluates the effect of commercially available and native AM fungi inocula on plant productivity in non-sterile post-agricultural soil. In a full-factorial manipulative greenhouse study, we assessed plant responses to water stress and inoculation of native, post-agricultural, and commercial AM fungi for application in arid post-agricultural restorations. We addressed two questions in this study: 1) does inoculation with either commercial or native AM fungi increase biomass of plants compared to AM communities already present in post-agricultural soil? and 2) do water-stressed plants respond more positively to native AM fungi?

Methods

Study system and field collection

Soil and AM fungi inocula were collected from two sites (native desert grassland and post-agriculture) in the San Luis Valley, Colorado, in August 2012. About 1L of soil was sampled from the root zone of plants to be used as sources of AM fungal inocula from 10 randomly located points per site located approximately 15m apart. Soil was stored at 4°C for less than two weeks until it was used for spore extraction. Native AM fungi inocula (Native) was collected from a desert grassland within the Nature Conservancy Preserve, Zapata Ranch (37°39'19"N, 105°35'11"W). The Zapata Ranch is located just west of the Sangre de Cristo Mountains and borders the Great Sand Dunes National Park and Preserve. Vegetation at this site consists of *Bouteloua gracilis*, *Stipa*

hymenoides, *Sporobolus airoides*, *Agropyron smithii*, *Krascheninnikovia lanata*, *Ericameria nauseosa*, *Kochia scoparia*, and *Atriplex canescens*. Post-agricultural AM fungi inocula (Control-2) and soil utilized in the greenhouse study (Control-1) were collected near Hooper, Colorado, on a 3-ha private field corner (37°43'41"N, 105°58'25"W) owned by Zapata Seed. The site had been irrigated for grain and potato production over 30 years ago. A single crop of potato minitubers had been planted and harvested over 10 years ago at this site. Since then, the site has been in annual weed cover of two non-mycotrophic species, *Kochia scoparia* and *Salsola kali*. Soil samples from both sites were sent to the Colorado State University Soils Laboratory for analysis. The post-agricultural site had higher levels of both N and P than the native site (Table 1C).

Preparation of Inoculum

To get an AM fungi sample representative of each site, soil samples within sites were thoroughly homogenized. Spores were extracted from twelve, 25g samples by sucrose centrifugation flotation (Allen et al. 1979) and counted under a dissection microscope to assess spore density (\pm SE/g soil). Mean spore density at the Zapata Ranch was 46 ± 2 /g and at the post-agricultural site was 76 ± 3 /g. Spores were rinsed thoroughly with diH₂O and transferred to a sterile test tube. Distilled water was added to spores to a final volume of 40mL and stored at 4°C until time of plant inoculation. Although AM fungi use vesicles from infected roots, extraradical hyphae, and spores to colonize roots (Klironomos and Hart 2002), we chose to use AM fungal spores so that we could control

variability in the quantity of inoculum and limit introduction of different abiotic factors or non-AM organisms that could occur from using a whole-soil inoculum (Ji et al. 2010). Usually, a restoration study that uses native AM fungi inoculants does not limit propagule sources to just fungal spores (Allen and Allen 1986). However, spores alone have been shown to be an effective means of colonization; and a study by Klironomos and Hart (2002) showed that all of 8 species of AM fungi tested were able to use spores to colonize roots.

Greenhouse design

Plants used in this study were chosen from species selected for use in a 16 thousand hectare restoration project in the San Luis Valley, Colorado. We used seeds from four different plant functional groups because this has been shown to be a good predictor of response to AM fungi inoculation (Hoeksema et al. 2010). Seeds of *Bouteloua gracilis* (blue grama, C4 grass), *Elymus lanceolatus* (thickspike wheatgrass, C3 grass), *Linum lewisii* (Lewis flax, forb), and *Medicago sativa* (alfalfa, N-fixing forb) were obtained from Western Native Seeds (Coaldale, Colorado). Species were seeded directly into separate deep tubes filled with non-sterile post-agricultural soil from the previously irrigated field corner. We used non-sterile soil from the field to better mimic soil conditions encountered in restorations. Seeds were watered daily until germination (about 6 days) and containers were then inoculated with approximately 120 spores by applying a 1mL aliquot to seedlings. Seedlings were thinned to maintain 3 plants per replicate. There were four different AM fungi treatments: 1) laboratory prepared local

inocula (Native); 2) commercially available inocula (Micronized Endomycorrhizal Inoculant, BioOrganics, New Hope, PA) (Industrial); 3) laboratory prepared post-agricultural inocula (Control-2); and 4) uninoculated non-sterile control (Control-1). To control for differences in the resident nonAM microbial community, and more accurately represent net effects of AM fungi inoculation on plants (Hoeksema et al. 2010) , a microbial washing was prepared by blending soil from each site and water in a 1:2 ratio (Johnson et al. 2010). The soil solutions were passed through a 45µm sieve to remove spores and other large soil organisms, but to retain microbes (Koide and Li 1989). Each container received a total of 40ml of sievate, 20ml from each field site.

After plants had germinated, two different water treatments were applied to seedlings: well-watered and water-stressed. The well-watered treatment received 20ml of water every three days maintaining soil moisture at field capacity. The water-stressed treatment received 20ml of water every 6 days. Plants were arranged randomly on greenhouse benches and supplemented with high pressure sodium lights to maintain 16 hours of daylight. Temperature was held at around 20°C for the duration of the experiment, which ran from November 2012 to March 2013 for a total of 16 weeks.

Plant harvest and root assessment

Plants were harvested after 16 weeks. Roots and shoots of each plant were dried and weighed separately. A small subsample (approximately 0.2g) of roots was taken from each plant prior to drying for fungal colonization analysis. Roots were cleared in 10% wt/vol KOH and stained using the vinegar-ink method (Vierheilig et al. 1998) using 5%

black Shaeffer ink. Root subsamples were assessed for AM fungi colonization percentage using the magnified gridline intersect method (McGonigle et al. 1990). Intersections were marked as positive for colonization if there was presence of hyphae, arbuscules, or vesicles. One-hundred intersections were viewed for each subsample.

Data Analysis

We examined the effects of host plant species, water treatments, AM fungi treatments, and their interactions on host plant biomass and root colonization using analysis of variance (ANOVA). Plant biomass and colonization data was log transformed to meet statistical assumptions. When significant results were found, means were compared using Tukey's Highly Significant Difference post-hoc tests. All statistical analyses were performed with JMP Pro 10.0.0 (SAS Institute Inc., Cary, NC).

Results

Root colonization

All plant roots examined were colonized with AM fungi, as evidenced by the presence of arbuscules and vesicles. Root colonization did not differ in response to the main treatment effects and there were no interactions between main effects (Table 2C). Colonization of roots was not correlated to aboveground biomass ($R^2 = 0.021$, $p = 0.098$)

Plant productivity

There was a different aboveground growth response between plant species and AM fungi treatment (Species x AMF treatment, Table 3C). Lewis flax had significantly reduced growth when inoculated with the native AM fungi compared to the uninoculated control (Tukey's HSD $\alpha < 0.05$). However, differences in growth within plant species were not significant as tested with Tukey's HSD for blue grama, thickspike wheatgrass, or alfalfa (Figure 1C). Plants in the water-stressed treatments had visible signs of stress including, wilt, leaf-curl, and death of 3 alfalfa plants. The water-stressed treatment caused significant reduction in total plant biomass. However, there was no interaction between AM fungi treatment and water treatment. Overall growth of plant species, regardless of water treatment, was highest in the Control-1 AM fungi treatment. There was no effect of mycorrhizal inoculation on root biomass, or root:shoot ratio.

Discussion

The major findings of this study were that: 1) the addition of native or commercial AM fungi to non-sterile post-agricultural soil did not increase plant productivity; 2) water-stressed plants did not respond differently to AM fungi inoculation; and 3) the addition of a native AM fungi reduced productivity in Lewis flax. The results from our study suggest that inoculation with commercial AM fungi does not provide greater benefits to growth when added to post-agricultural soil than the resident AM fungi. The native AM fungi treatment elicited a more nuanced growth response from blue grama and Lewis flax plants. Surprisingly, the native AM fungi treatment was not more effective

when plants were water-stressed. Marulanda et al. (2007) found a native AM fungi community more effective at alleviating drought stress in plants. We did not observe this in our experiment and there was a tendency for greater growth in our un-inoculated control AM fungi treatment.

Higher spore densities were found in the post-agricultural soil compared to the native field site, which agrees with others who compared similar systems (Johnson et al. 1991, Richter et al. 2002). By disrupting hyphal networks, agricultural disturbances may select for fungal species that infect and proliferate mainly by spores (Johnson et al. 1992), explaining the high numbers found in our post-agricultural soil. Spores in native arid grasslands, however, have been shown to be distributed heterogeneously suggesting that hyphal networks are more important for colonization than spores in undisturbed systems (Richter et al. 2002). Therefore, by limiting native AM propagules to just spores in our experiment, we may have lowered the inoculum potential of our native AM fungi treatment. We did not find differences in colonization rates between treatments, which agrees with findings of Richter et al. (2002), who found no differences in colonization rates between post-agricultural and native grassland AM fungi inocula in Arizona. Colonization of AM fungi was not correlated to plant growth in our study, and colonization does not always translate to plant productivity and vice versa (Requena et al. 1996, Klironomos 2000, 2003). This is especially true if AM colonizers are less mutualistic (Johnson et al. 1992) and drain carbon from their host plants. However, our staining technique made observation of fungal structures difficult and our inclusion of fungal hyphae may have over-estimated or obscured actual colonization rates. We do not

know if colonization was due to resident AM fungi in the post-agricultural soil or due to inoculated AM fungi. Since there were different patterns of response to AM fungi treatments, it may be that inoculation had some, albeit small, effect on plant biomass.

Foreign or industrially produced inocula may not be appropriate when trying to increase native plant growth, or diversity in restorations. Commercial AM fungi species are selected and produced based on their ability to quickly propagate (Schwartz et al. 2006), but unfortunately, this does not mean that these fungi reflect interactions that would occur in natural AM-plant interactions (Klironomos 2003). Nor does it mean that they will persist in the soil once they have been introduced (Requena et al. 2001). In our study, industrial inocula did not confer greater growth benefits to plants over the resident post-agricultural AM fungi community. Our results corroborate findings by White et al. (2008), whose study showed that plant community and growth were not affected by a commercial AM fungi inoculation in a roadside restoration, and also tested inoculation in a soil with high levels of phosphorus. Other studies have also demonstrated the ineffectiveness of commercial AM fungi to colonize montane plant roots in Colorado (Rowe et al. 2007), or to confer growth benefits to prairie grasses in post-agricultural soil (Paluch 2011). Our results, together with other studies, demonstrate that the cost of using industrial AM fungi inocula in addition to the high costs already associated with arid restoration (Allen 1995) may not be worthwhile.

Plant community composition can be altered by the presence of certain AM species or the composition of AM communities (Van der Heijden et al. 1998b, Klironomos et al.

2000, Rillig 2004). A plant's responsiveness to AM fungi also varies depending on the plant's functional group (Wilson and Hartnett 1998) or associated successional stage (Collins and Foster 2009, Middleton and Bever 2012), and the particular AM fungi taxa or AM fungi origin (Van der Heijden et al. 1998a, Klironomos 2003). Because of the complexity of possible interactions among these symbionts (Klironomos 2003), it is not surprising that there was an interaction between plant species and AM fungi treatments in our study. Our native AM treatment had positive effects on the growth of blue grama and tended to negatively affect early seral species, which might suggest its usefulness as an inoculation source to facilitate succession by favoring later seral species. Other studies have demonstrated benefits of using native soil inoculum sources to advance succession in post-agricultural restorations (Middleton and Bever 2012) or in other high P soil environments (Rowe et al. 2007). However, our results may also suggest that the native AM fungi reduce diversity, or perhaps overall productivity, by its benefits to only blue grama. This disagrees with findings from Klironomos (2003), who suggested that native fungal inoculants are more important determinants for plant diversity than foreign inoculants of the same AM species (Klironomos 2003). Caution should be taken when extrapolating our results to larger ecosystems, as AM fungi may not behave similarly in the field as they do in controlled greenhouse experiments (Johnson and Pflieger 1992). Complicated plant-soil biota interactions can drastically alter our observed effects in complex communities (Callaway et al. 2004, Hoeksema et al. 2010).

Arbuscular mycorrhizal fungi can have many different roles over the course of their lives (Brundrett 2004), which range from mutualistic to parasitic (Johnson et al. 1997,

Klironomos 2003). Whether host plants benefit from the symbiosis depends on a variety of factors, among which is the availability of nutrients in the soil (Hoeksema et al. 2010). In highly fertile soil, such as our post-agricultural soil, facultative biotrophic plants need not rely upon fungal associations for nutrient uptake. As a result, the fungi can act antagonistically, draining carbon from their host plants (Johnson 1993, Johnson et al. 1997, Brundrett 2004). However, most AM fungi symbioses are balanced, a special type of mutualism that wavers in time by shifting benefits to one partner over the other (Brundrett 2004). Although the association of our native AM fungi on plant hosts in our study appears to be antagonistic (with the exception of blue grama), it may be that the short time studying these plants was only a snapshot of their relationship (Brundrett 2004). In a re-vegetation experiment in Spain, plants did not respond well to a native AM inoculant in the first sampling year (Requena et al. 2001). However by the fifth year, plants inoculated with native AM fungi were nearly twice as large as plants uninoculated or inoculated with a foreign AM fungi. Our plants may have responded differently had sampling extended over a longer period.

Inoculation of AM fungi may confer other benefits that are not considered when only examining plant biomass or percent cover. For example, in a revegetation experiment in Wyoming, inoculation did not result in higher percent cover of species planted, but inoculated plants experienced less competition from annuals during periods of drought (Allen and Allen 1986). Also, Requena et al. (2001) demonstrated that inoculation with a native AM fungi community increased soil structure in a degraded shrubland in Spain. Future work on best management practices of inoculation treatments in restoration

(Schwartz et al. 2006) may want to test different levels of inocula in mesocosms consisting of more diverse plant mixes, with replicates of successional species, or test other benefits of inoculation besides effects on plant growth.

Another area of ecology gaining importance in restoration is the study of community assembly and priority effects. Plants have been shown to exhibit strong priority effects that have lasting effects on community composition (Körner et al. 2008, Collinge and Ray 2009, Wainwright et al. 2012). It might be that our inoculations did not have more dramatic effects, because they were experiencing competition from the resident post-agricultural AM fungi via priority effects. It might be that more frequent introductions of AM fungi would increase the effect of AM fungi on plant growth. Species of AM fungi have been shown to be associated with different successional stages and there are functional groups of AM fungi (Renker et al. 2004). However, to our knowledge, there are no studies investigating how priority effects might change the composition of AM fungal communities. This may be an area for future investigation on AM fungi in restoration. It may help to explain some of the ambiguous results found in restorations in areas of non-sterile soils.

Conclusion

Results from restoration projects investigating manipulation of AM fungi have been ambiguous (Harris 2009). Given the difficulty and cost of introducing AM fungi inocula, it may not be a worthwhile restoration strategy, especially when phosphorous levels are high in the soil (White et al. 2008) as in the post-agricultural soil used in our

study. Inoculation should not be ruled out completely, but care should be taken when implementing treatments in the field. Our study confirms the need to test AM fungi inoculation before any use in large-scale restoration projects.

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APPENDIX A

Table 1. Summary of restoration studies investigating irrigation treatments in semiarid and arid grasslands

Reference	Location	System ^a	Species ^b	Planting Method ^c	Irrigation ^d	Total Irrigation	Planting Date ^e	Precip ^f	Results
Banerjee et al. (2006)	Arizona	PA	S, G, F	S	SI – 15 mo.	100mm	D	D	Irrigation increased natives (cover still only <4%) weeds dominated plots
Ries et al. (1988)	North Dakota	PA	G, F	S	SI – 2yrs	47 - 697mm	SU, D	W	All water treatments increased seeded species and decreased weeds
Roundy et al. (2001)	Arizona	PA	S, G	S	PI – 10 days	280.5mm	SU	N/A	Irrigated plots had earlier germination and higher survival.
Padgett et al. (2000)	California	PA	S	S	SI	>1000mm	D	D	Irrigation decreased diversity. Natural precipitation was sufficient for establishment.
James and Svejcar (2010)	Oregon	DR	G, F	S	SI – 2 mo.	80mm	D	D	No difference in irrigated and control plots.

Zimmermann et al. (2008)	Zambia	ER	G	S	SI – 1 yr.	166mm	S	A	Irrigation improved early stages of growth, but not overall recruitment.
Yamashita and Manning (1995)	California	PA	S	T	SI – 2yrs	20L	D	D	Survival increased with irrigation. Growth increased with irrigation in first year.
Belnap and Sharpe (1995)	Utah	PR	G	S	SI – 8mo.	N/A	D	D	Irrigation had no effect on natives compared to control, but biomass of exotics increased 70% when watered.

a System. PA = Post agricultural, DR = Degraded rangeland, ER = Experimental rangeland, PR= Park Reserve

b Functional groups planted. S = shrubs, G = grasses, F = herbaceous forbs, T = Trees

c Planting methods. S = seeded, T = Transplant

c Irrigation treatment applied. PI = only during planting, SI= applied and subsequently thereafter

d Season of planting or sowing. S = spring, SU = summer, fall/dormant = D

e Precipitation compared to average at time of seeding. W = wet, D = Dry, A = average

Table 2. Summary of restoration studies investigating AM fungi treatments in restoration

Reference	Location	System ^a	Species ^b	Planting Method ^c	Soil Characteristics	Inoculum ^d	Sampling period	Survival	Growth
Caravaca et al. (2003)	Spain	DR	S	T	low-nutrient, non-sterile	F(I), NA(M)	1 yr	No difference	Increased by both inoculations. Native slightly better.
Middleton and Bever (2012)	Indiana	PA	G, F	T, S	N/A, non-sterile	WN	3 yrs	1 out of 4 nurse plants improved	Positive effect on later successional species. Negative effects on early successional species.
Paluch (2011)	Wisconsin (Greenhouse)	PA	G, F	S	N/A, non-sterile	IN (M)	83 days	No effect	No effect
Carbajo et al. (2011)	Netherlands (Greenhouse)	PA	G, F	S	High-nutrient and low-nutrient, non-sterile	WN	4 mo.	N/A	Increased diversity. Improvement less pronounced in organic vs. mineral soil.
Requena et al. (2001)	Spain	DS	S	T	N/A, non-sterile, low density native AM	F(I), NA(M)	5 yrs	Increased	Increased biomass of plants twofold compared to foreign.
Rowe et al. (2007)	Colorado (Greenhouse)	PR	S, G, F	S	High-nutrient, non-sterile and sterile	WN, IN(I), IN(M)	About 100 days	N/A	(WN) positive effects on late seral species, negative on early. (F) ineffective at colonizing roots

White et al. (2008)	Minnesota	DR	G, F	S	High P	NA (M), IN (M)	27 mo.	N/A	Neither treatment differed from control of total or desired biomass
Allen and Allen (1986)	Wyoming	MI	S, G, F	S	Same as native soil	WN	15 mo.	N/A	No increase. In some cases decreased cover. Increased competitive ability during drought.
Smith et al. (1998)	Minnesota	DR	G, F	S	Recipient site lower in P than donor site	WN	1 year	N/A	Increased cover of natives.

a System. PA = Post agricultural, DR = Degraded rangeland, DS = Degraded shrubland, PR= Park Reserve, MI = Mined

b Functional groups planted. S = shrubs, G = grasses, F = herbaceous forbs, T = Trees

c Planting methods. S = seeded, T = Transplant

d Fungal inoculum used individually (I) or in mixes (M). NA = Native, IN = Industrial, F = Foreign, WN = whole-soil native

APPENDIX B

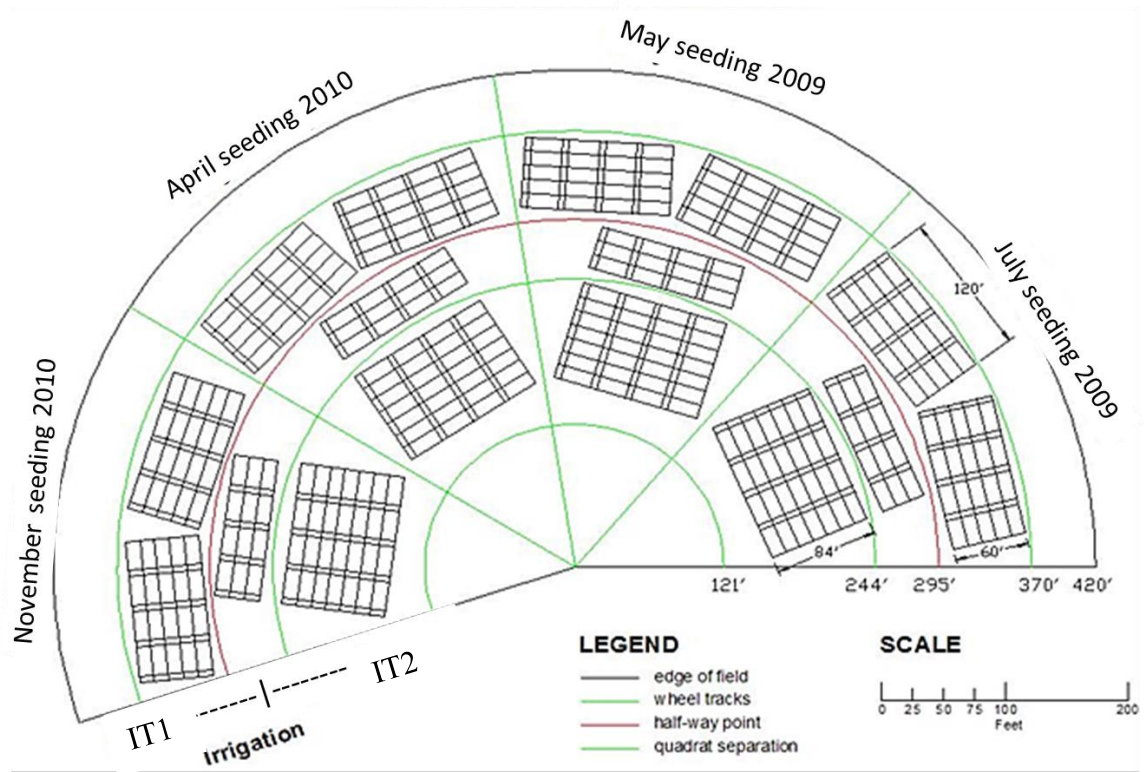


Figure 1. Experimental layout of abandoned field corner in Hooper, Colorado. Outer plots located past “half-way point” received IT1 irrigation. Plots located inside the “half-way point” were treated with IT2 irrigation.

Table 1. Species list of plants drill-seeded in individual species plots and mixed species plots of 3-ha retired field corner in San Luis Valley, Colorado.

<i>Scientific Name</i>	<i>Common Name</i>	<i>% of mixed species treatment</i>
Native Grasses (Single)		
<i>Oryzopsis hymenoides</i>	Indian ricegrass	30.9
<i>Bouteloua gracilis</i>	Blue grama	7.7
<i>Elymus elymoides</i>	Bottlebrush squirreltail	11.8
<i>E. lanceolatus</i>	Streambank wheatgrass	9.9
<i>E. lanceolatus</i>	Thickspike wheatgrass	9.2
<i>Pascopyrum smithii</i>	Western wheatgrass	12.5
<i>Sporobolus airoides</i>	Alkali sacaton	5.1
<i>S. cryptandrus</i>	Sand dropseed	0.7
Non-Native Grasses (Mix)		
<i>Agropyron cristatum</i>	Crested wheatgrass	0
<i>Psathyrostachys juncea</i>	Russian wildrye	0
Forbs Mixture		
<i>Adenolinum lewisii</i>	Lewis flax	2.2
<i>Artemisia ludoviciana</i>	Louisiana sage	0.4
<i>Atriplex canescens</i>	Fourwing saltbush	1.1
<i>Ceratoides lanata</i>	Winterfat	2.2
<i>Medicago sativa</i>	Alfalfa	2.2
<i>Melilotus officinalis</i>	Yellow sweetclover	1.8
<i>Ratibida columnifera</i>	Prairie coneflower	1.1
<i>Kochia prostrata</i>	Forage kochia	1.1

Table 2. Irrigation (mm) applied in 2009, 2010, and 2011 on 3-ha field corner.

Seeding Date/Year	2009	2010	2011	Total
May IT2	208	99	84	391
May IT1	269	142	46	457
July IT2	173	99	84	356
July IT1	193	142	46	381
Nov IT2	0	302	0	302
Nov IT1	0	437	46	483
April IT2	0	302	0	302
April IT1	0	437	46	483

Table 3. Soil analyses of planting dates May and July, and IT1 and IT2 irrigation treatments. Soils were sampled in November 2012 and represent homogenized composites of 5 randomly sampled points in each treatment.

Sample	pH	EC Mmhos /cm	% OM	ppm							meq/L				SAR	Texture
				NO ₃ - N	P	K	Zn	Fe	Mn	Cu	Ca	Mg	Na	K		
May-IT1	8.1	0.4	0.7	6.8	15	347	1.0	2.1	2.6	1.3	1.2	0.4	0.4	0.8	0.4	Sandy Loam
May-IT2	8.0	0.4	0.8	10.5	18	334	1.1	1.9	3.2	1.3	1.7	0.5	0.4	0.7	0.4	Sandy Loam
July-IT1	8.2	0.4	0.8	4.4	16	343	0.84	1.8	3.0	0.78	1.1	0.3	0.4	0.8	0.5	Sandy Loam
July-IT2	8.1	0.5	1.0	9.5	25	464	0.97	1.8	3.3	1.1	1.3	0.4	1	1.2	1.1	Sandy Loam

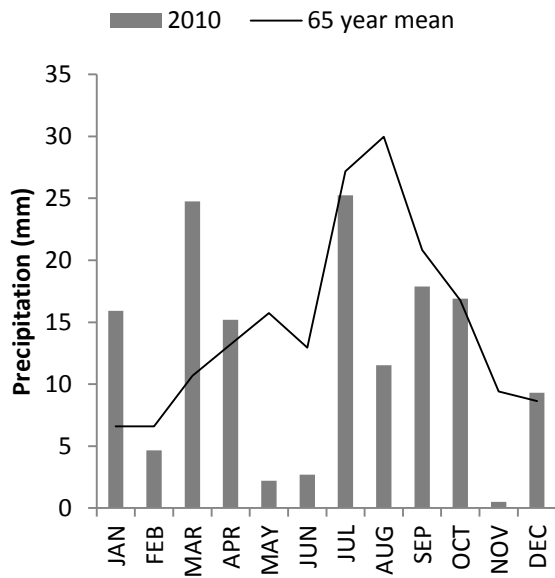
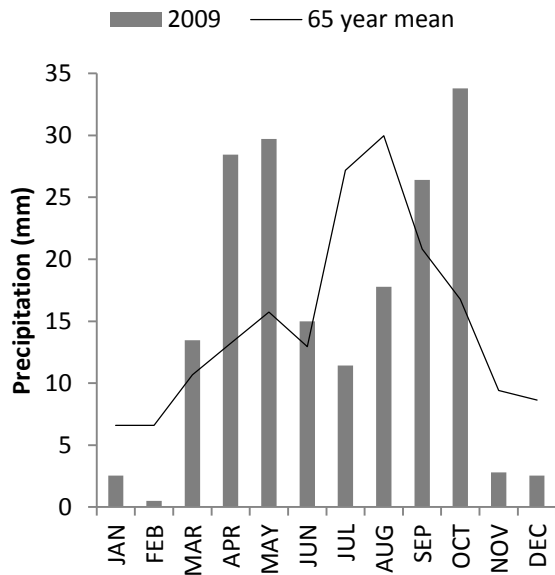


Figure 2. Precipitation data from 2009 and 2010 in the San Luis Valley, Colorado. Data were collected from the nearby Alamosa weather station and obtained from the Western Regional Climate Center.

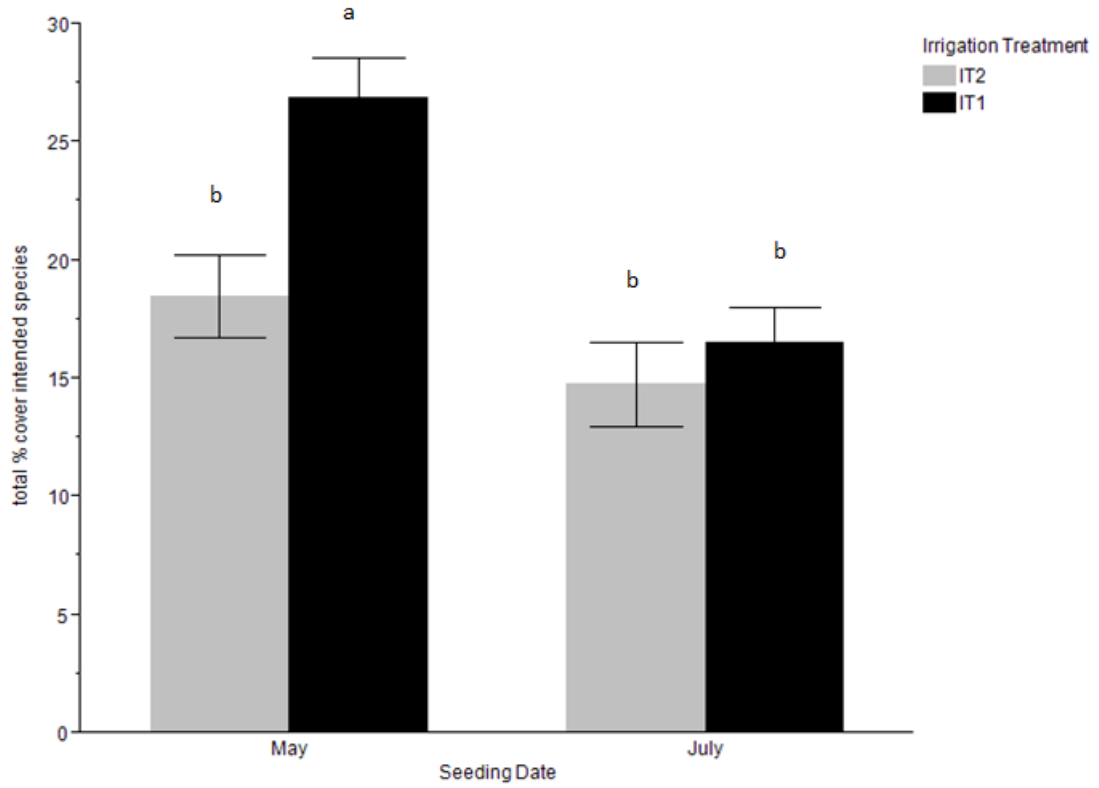


Figure 3. Planting date (May, July) and irrigation treatment (IT1, IT2) combinations on relative ground cover for intended species sampled in August 2012. Bars represent means and standard errors of all species averaged for each treatment. Different letters over bars indicate significant difference reflected by Tukey’s HSD post hoc test at $\alpha < 0.05$.

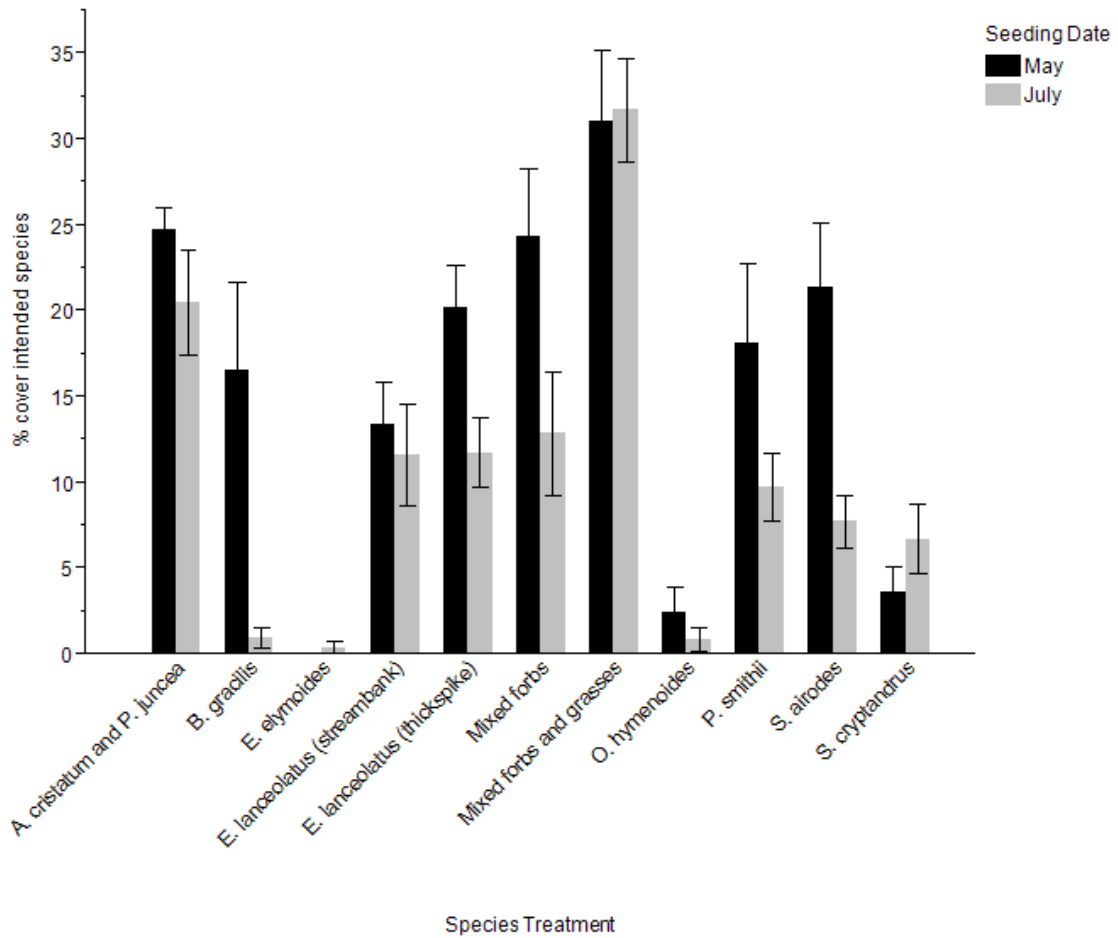


Figure 4. Effect of seeding date x species treatment on percent ground cover of intended species sampled in August 2012, after irrigation had ceased. Bars represent means averaged over irrigation treatments. Error bars represent ± 1 SE.

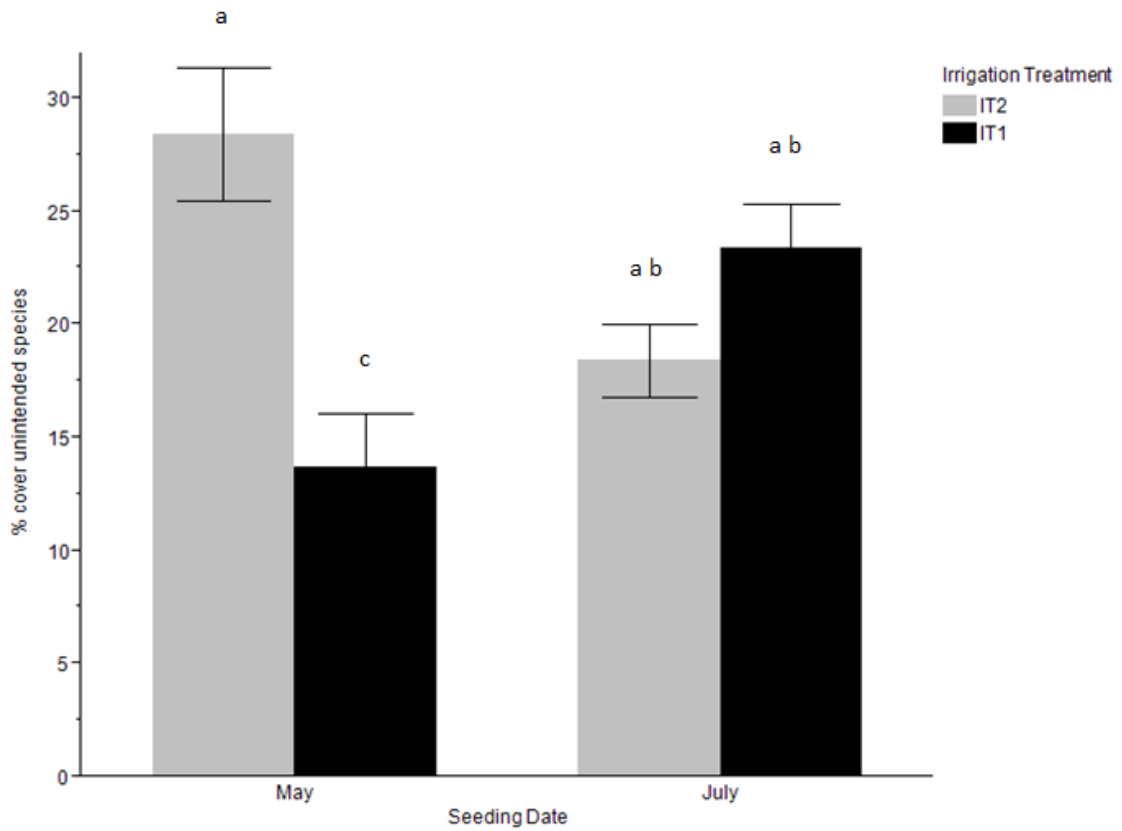


Figure 5. Planting date (May, July) and irrigation treatment (IT1, IT2) combinations on relative ground cover of unintended species sampled in August 2012. Bars represent means and standard errors of all species averaged for each treatment. Different letters over bars indicate significant difference reflected by Tukey's HSD post hoc test at $\alpha < 0.05$.

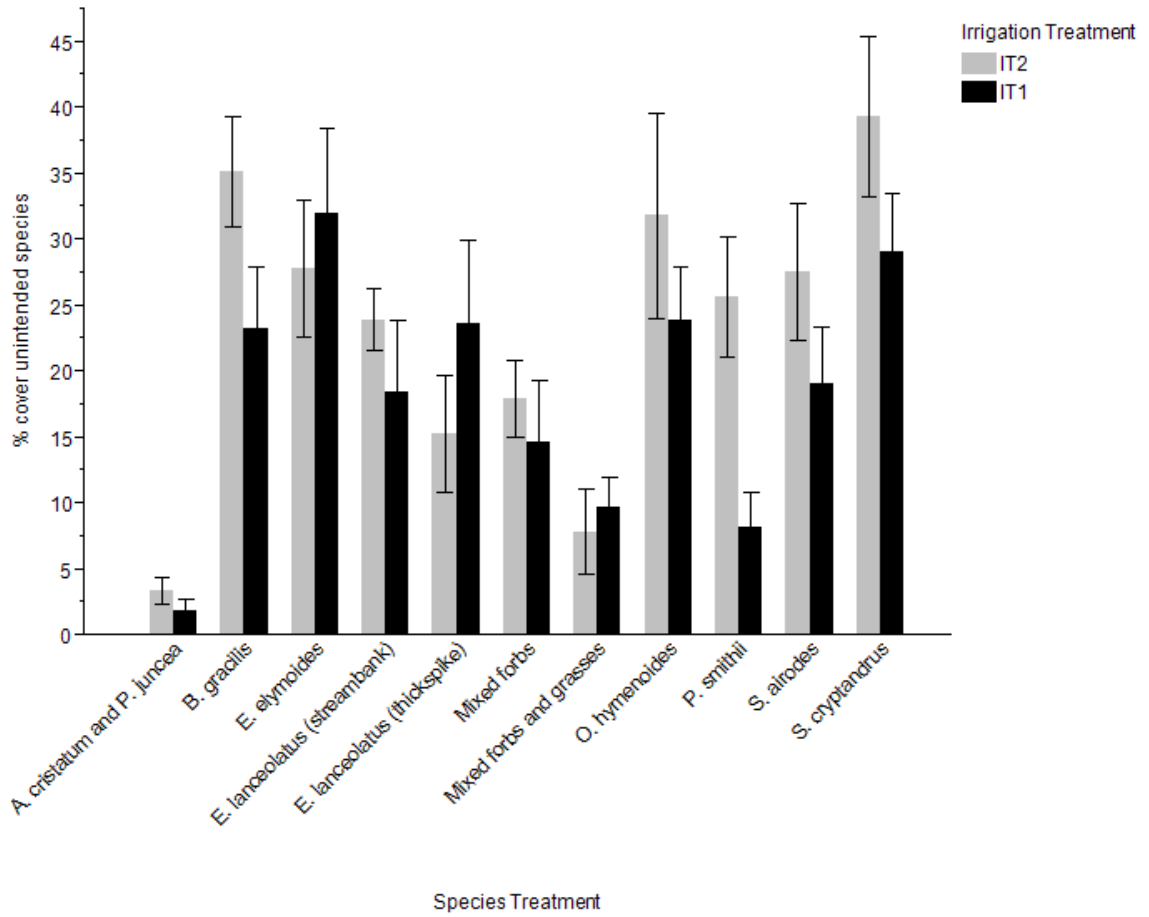


Figure 6. Effects of irrigation treatment x species treatments on percent ground cover of unintended weed species sampled in August 2012 after irrigation had ceased. Bars represent means averaged over planting date treatments. Error bars represent $\pm 1SE$.

APPENDIX C

Table 1. Soil analyses of native desert grassland (Zapata), the donor site for AM fungi spore extraction, and post-agricultural soil used for Control-2 spore extraction and as planting media.

Source	pH	EC mmhos/cm	OM	NO3-N (ppm)	P (ppm)	K (ppm)	Texture
Zapata Ranch	7.2	0.5	0.9%	7.9	11.5	361	Sandy Loam
Post- Agriculture	7.8	0.7	1.0%	10.6	25.0	995	Sandy Loam

Table 2. Results from ANOVA on the effect of water treatment, plant species, AM fungi treatments and their interactions on root colonization

Colonization	DF	F ratio	P value
Species	3	1.38	0.254
AMF treatment	3	1.42	0.241
Species*AMF treatment	9	1.02	0.426
Water Treatment	1	0.0063	0.936
Species*Water Treatment	3	1.75	0.161
AMF treatment*Water Treatment	3	0.99	0.401
Species*AMF treatment*Water Treatment	9	1.19	0.309
Error	98		
Model	129	1.17	0.277

Table 3. Results from ANOVA on the effect of water treatment, plant species, AM fungi treatments and their interactions on host plant aboveground biomass

Shoot	DF	F ratio	P value
Species	3	831.07	<0.0001
AMF treatment	3	3.69	<0.05
Species*AMF treatment	9	2.15	<0.05
Water Treatment	1	102.19	<0.0001
Species*Water Treatment	3	8.98	<0.0001
AMF treatment*Water Treatment	3	0.48	0.694
Species*AMF treatment*Water Treatment	9	0.58	0.810
Error	31		
Model	188	88.1872	<0.0001

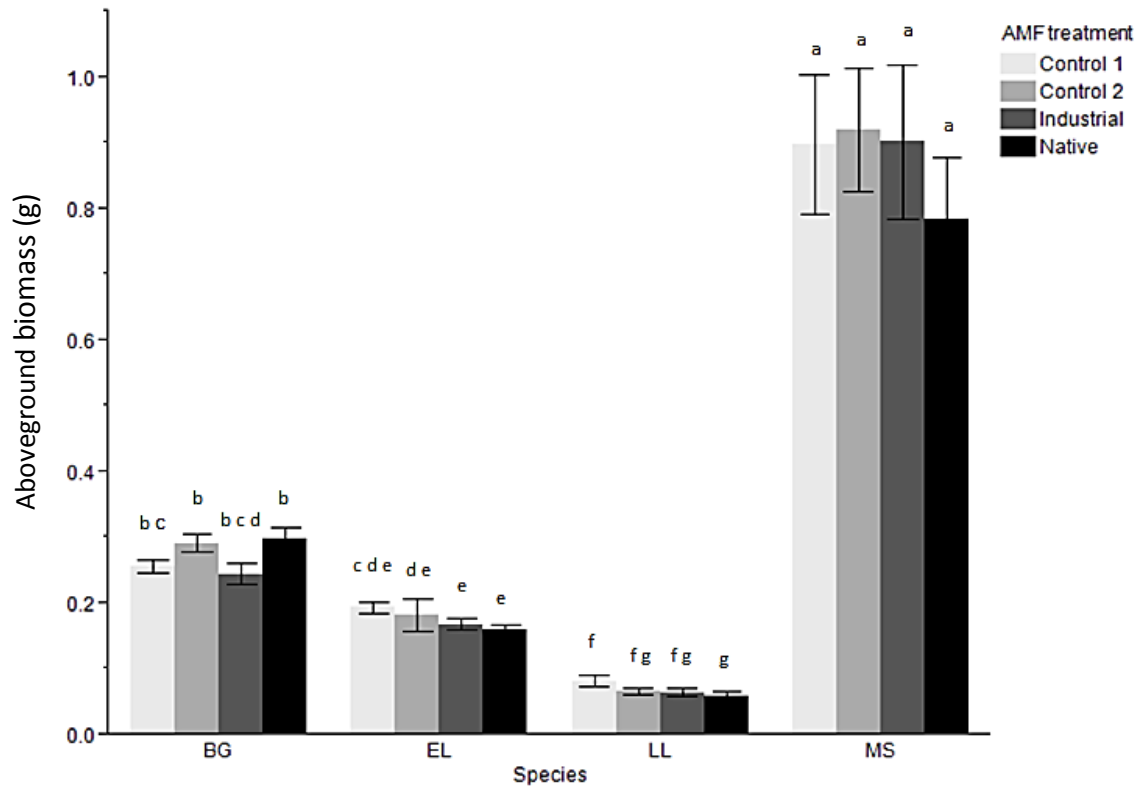


Figure 1. Effects of AM fungi treatments Control 1 = uninoculated soil, Control 2 = laboratory prepared post-agricultural AM fungi, Industrial = commercially available AM fungi inoculant, Native = laboratory-produced native AM fungi on shoot biomass (g) of BG = blue grama, EL = thickspike wheatgrass, LL = Lewis flax, MS = alfalfa. Error bars represent ± 1 SE. Different letters over bars represent significant differences between means (Tukey's HSD $\alpha < 0.05$).