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The effects of mycorrhizal inoculant and micronutrients on early plant establishment during a tallgrass prairie reconstruction

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THE EFFECTS OF MYCORRHIZAL INOCULANT AND MICRONUTRIENTS
ON EARLY PLANT ESTABLISHMENT
DURING A TALLGRASS PRAIRIE RECONSTRUCTION

An Abstract of a Thesis
Submitted
In Partial Fulfillment
Of the Requirements for the Degree
Master of Science

Christopher L. Barber
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May 2018

ABSTRACT

Symbiotic relationships between mycorrhizal fungi and land plants are one of the most widespread examples of symbiosis on Earth yet there is still much to discover about their ecological impacts.

Prairie reconstructions are often done on highly disturbed sites such as reclaimed cropland, turf grass, and road rights-of-way. Disturbed soils often lack adequate quantities of both mycorrhizal fungi and micronutrients. I hypothesized that inoculated seeds with mycorrhizal spores or micronutrient seed treatments will aid in the early establishment of a tallgrass prairie.

To test this hypothesis four treatments (T1 control, T2 mycorrhizal, T3 micronutrient, and T4 mycorrhizal and micronutrient) were planted with a seed mixture of 36 native species (8 grasses and 28 forbs). This was a split block experiment with three replicates in each block, and individual plot size was 15 m². Vegetation was examined using seedling count, basal coverage, above ground biomass, inflorescence count, and mycorrhizal colonization percentage.

There was a significant increase ($p=0.006$) in total native seedlings in the mycorrhizae plots over the control plots was seen in Year 1. In Year 2 increases of 51.7% ($p=0.000$), 41.5% ($p=0.001$), and 45.1% ($p=0.000$) in total native basal coverage were seen for the mycorrhizae, micronutrient, and combination treatments respectively over the control.

Neither the number of weed seedlings in Year 1 nor the basal coverage of weed species in Year 2 was significantly different among any of the treatments. Basal coverage of weeds was reduced in Year 2 though it was only marginally significant ($p=0.102$).

In Year 2 there was significantly ($p=0.001$) higher native biomass in in all treatments versus the control. Weed biomass was not significantly different although overall weed pressure was low and there was a high degree of variability in the data.

Mycorrhizae sampling revealed that there was approximately 42% ($p=0.000$) increase in mycorrhizal colonization in the treatments that had mycorrhizal inoculant added to the seed mixture than the plots that did not receive inoculant.

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This Study by: Christopher Barber
Entitled: The Effects of Mycorrhizal Inoculant and Micronutrients on
Early Plant Establishment During a Tallgrass Prairie Reconstruction
Has been approved as meeting the thesis requirement for the
Degree of Master of Science

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DEDICATION

For my family, Gracie, Hazel, and especially Kayla whose sacrifices,
support and encouragement made this possible.

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CHAPTER 1

INTRODUCTION

The state of Iowa is located in the heart of the historic range of tallgrass prairie which covered the vast majority of the state's landscape (Smith et al. 1998). Over the past 200 years this landscape underwent a dramatic transformation from a natural ecosystem to one dominated by row crop agriculture or constructs of post-settlement society. Currently, conservationists and land managers put great effort and resources into protecting existing prairie and reconstructing new prairie habitat when the opportunity presents itself.

However, there are numerous barriers that prevent a more aggressive expansion of prairie reconstruction efforts. The basic ingredients for a prairie reconstruction are prairie seed, land availability, a labor force, and equipment; all of which require significant amounts of money. Therefore, it is in a land manager's best interest to implement reconstruction practices that are both ecologically sound and financially prudent. This highlights the importance of restoration and reconstruction experiments that expand existing scientific knowledge and provide practical guidelines for real-world land management.

This study investigates the effects of a biotic, mycorrhizal fungi, and an abiotic, micronutrients, soil property enhancement on the

establishment of tallgrass prairie vegetation. It has been estimated that mycorrhizal-plant relationships occur in 70-90% of all plant species (Brooks et al. 2006). There are two types of mycorrhizae: ectomycorrhiza and endomycorrhiza. Ectomycorrhiza form a hyphal sheath over the exterior of the root tips and only colonize woody-stemmed plant species. Conversely, endomycorrhiza have hyphae that grow into the root tissue of herbaceous plant species. Both fungi types serve a generally similar role by facilitating nutrient flow from the soil into the root tissue.

The role of arbuscular mycorrhizal fungi (AMF) in tallgrass prairie ecosystems of the Upper Midwest is still poorly understood. The traditional and simplistic explanation of how AMF facilitate plant growth is that AMF increase the amount of water and nutrients to their hosts in exchange for carbon in the form of sugars. This relationship is typically thought of as symbiotic in nature. However, as we learn more about the complexity of AMF-plant interactions, researchers have realized that physical and biological environmental factors dictate where the relationship falls on the cost-benefit continuum. Gaining knowledge about the function of AMF in natural environments will allow us to determine if there are implications for the restoration, reconstruction, and management of tallgrass prairie ecosystems.

Arbuscular mycorrhizal fungi are obligate biotrophs that require a host plant for growth and reproduction. An asexual fungal spore

germinates and grow for 1 to 3 weeks when the appropriate physical conditions exist, but growth stops before the spore reserve is depleted if no host root is available (Buee et al. 2000). If contact with a host root is made, AMF form fibrous root-like hyphae that enter the roots through the epidermal cells and form tree-like structures arbuscules within the cortical cells. The arbuscules are the interface for water and nutrient transfer which allow fungi to obtain carbon from the plant (Bever et al. 2001). In return, the fungi transfer water and nutrients into the root cells which can then be utilized by the plant (Brooks et al. 2006).

Fossil records indicate that arbuscular mycorrhizae-like fungi have been present on Earth since the Ordovician period between 455 and 460 million years ago (Redecker et al. 2000). This places the arrival of AMF before that of vascular plants which suggests a possible coevolution since the first plants on land did not possess a root system to take up water and nutrients. Although it may appear that a coevolution with land plants was likely, the fossil record can give no indication as to whether it was driven by symbiosis or competition in an evolutionary arms race. The available literature seems to suggest that environmental conditions and plant physiology dictate the current status of their relationship (Entry et al. 2002; Johnson et al. 1992).

Another piece of evidence supporting the theory of symbiosis is the presence of chemical signals being passed between plant and fungi

during the germination of fungal spores, attachment of the hyphae to the root, and plant responses to colonization (Harrison 2005). Buee et al. (2000) discovered that there is a root factor that stimulates growth and branching in fungal hyphae, but they had yet to uncover the chemical characteristics of the factor. Increasing the amount of branching increases the hyphae's chance of coming into contact with the root tissue and therefore the likelihood of fungal colonization. It has also been shown that plants grown in soil with low phosphorus levels exude more of the compound than plants that have adequate phosphorus availability (Harrison 2005). Hartnett et al. (1993) showed that phosphorus availability can be a determining factor to the extent that AMF will colonize a root. Navazio et al. (2007) found that AMF spores release signaling molecules that induce an increase in cytosolic calcium which is known to be a common component in chemical signaling. This demonstrates that there is signaling occurring in both the fungi and the plant providing further evidence in support of a coevolution between plant and fungi.

Mycorrhizal colonization can positively impact a plant by increasing its uptake of the essential nutrients (N, P, K, Ca, Mg, and S), micronutrients (Co, Cu, Mn, Mo, Zn, etc.), and water. It has been estimated through a greenhouse study that the nutrient uptake by the hyphae of AM fungi can supply up to 80% of the phosphorous and 25%

of the nitrogen required by an individual plant (Wilson et al. 2001). Liu et al. (2000) found that uptake of the micronutrients zinc, copper, iron, and manganese was increased in pot-grown maize infected by commercially produced mycorrhizal inoculant in sand and sandy loam medium. Nitrogen fixing legumes have also been shown to have decreased survivorship when mycorrhizal fungi are not present in prairie microcosms grown in a greenhouse (Wilson and Hartnett 1997).

Mycorrhizal colonization has been shown to increase drought resistance through the increased uptake of phosphorus which is used to help build new tissue (Nelson and Safir 1982). Water content in the cells of a mycorrhizal plant is increased as a mechanism of osmotic pressure regulation, thus more water is held in the cells of an infected plant making the plant more drought resistant (Auge 2001). When mycorrhizae are present, plant mortality during unseasonably dry periods should be reduced through the competitive advantages gained by colonization. This should help increase the rate that early successional and invasive species are shaded out by later successional species when mycorrhizae are present.

It has been shown that AMF can regulate and compete with other soil microorganisms in the rhizosphere. However, the mechanisms and pathways in which this occurs are still poorly understood. Fitter and Garbaye (1994) demonstrated that mycorrhizae can either inhibit the

function of pathogenic bacteria, fungi, and nematodes or promote beneficial bacteria such as those found in the root nodules of legumes. This should be another mechanism in which mycorrhizae increase plant survivorship as well as increasing atmospheric nitrogen fixation in legumes. Improving our understanding of these interactions will help in determining the role mycorrhizal fungi play in natural ecosystems.

The presence of AMF can also have profound effects on plant biomass, which can be used as an indicator of overall health and reproductive success depending on the physiology of individual species. *Vicia faba* L. (fava bean) has been observed to experience an increase in growth yield when AMF was present and an even greater increase when both AMF and *Rhizobium* were present (Jia and Gray 2008). Wilson and Hartnett (1997) found that warm-season C₄ grasses experienced a 31% increase in biomass while the cool-season C₃ grasses had a decrease in biomass. Forb biomass varied among individual species due to their level of mycorrhizal dependence and colonization rate. Growth responses to AMF colonization varies by species. A study by Wilson and Hartnett (1998) looked at the growth difference between colonized and uncolonized individuals of 36 grass species and 59 forb species that are found in tallgrass prairies in Kansas. Their results showed that growth and percentage of root colonization varied widely among forbs as well as confirming their previous findings that C₄ grasses gain an advantage over

C₃ grasses when AMF were present. The study also showed that colonization reduced the growth of certain non-native grasses such as *Bromus inermis* (smooth brome), *Bromus japonicas* (Japanese brome), and *Poa pratensis* (Kentucky bluegrass).

The benefits of mycorrhizal colonization in plants do not come without costs. As discussed by Bever et al. (2001) the direct cost to the plant is the loss of carbon in the form of sugar which was made during photosynthesis. During photosynthesis energy in the form of light and adenosine triphosphate (ATP) is required, in addition to water and carbon dioxide, to produce sugars needed for growth. If the costs of AMF colonization (i.e. carbon) outweigh the benefits received then the fungi act as a parasite and not as a symbiont (Paszkowski 2006). In the theoretical event that costs and benefits of colonization are equal, then the plant-fungal relationship would result in a commensalism because it is assumed that there is essentially no cost to the fungi to infect its host. It is important to remember that a plant's location on symbiotic-parasitic scale depends on that species' susceptibility to AMF infection as well as environmental factors (Johnson et al. 1997). They found that the most common cases of parasitism occurred when nutrient levels were increased through fertilizer or light levels were decreased.

There are indications that AMF may be used to enhance tallgrass prairie plant establishment and survival in restoration and

reconstruction projects (Smith et al. 1998, Requena et al. 2001). When natural ecosystems such as prairies go through a large-scale disturbance the biotic and abiotic characteristics of the soil are modified and become degraded. Prairie reconstructions are usually done in areas that have experienced a great deal of disturbance through compaction, herbicides and pesticides, grading, tilling, over-fertilization, and chemical contamination from runoff. AMF have been shown to help remediate many of these conditions such as compaction, high metal content, and the degradation of chlorinated phenolic compounds resulting from herbicide usage and industrial waste (Entry et al. 2002).

AMF inoculation has already been used in a variety of other ecosystems and has shown to be effective in restoration and reconstruction efforts. Requena et al. (2001) performed a long-term restoration experiment in a desertified Mediterranean ecosystem in southeastern Spain using AMF. They found inoculating with native AMF not only increased plant growth and survivorship of native seedlings, but it also increased soil nitrogen, soil organic matter, and hydrostable soil aggregates.

Greipsson and El-Mayas (2000) found that AMF inoculation increased seedling growth of the native dune grass *Leymus arenarius* (lymegrass) when planted in coastal sand dunes along the coast of Iceland. The low nutrient levels of sand dunes are an example of an ideal

candidate for the use of mycorrhizal inoculant in restoration. Although there has been much less data collected on wet and mesic ecosystems, Turner and Friese (1998) found AMF present in a wet prairie/fen ecosystem in Ohio. This shows that AMF naturally occur in wet ecosystems and they have a natural function within that ecosystem.

Since AMF have varying effects on different species, they have the potential to influence community structure and diversity in an ecosystem. Obligate mycotrophs will gain a competitive advantage over species closely proximal that are less dependent on mycorrhizae. Hartnett et al. (1993) found that during interspecific competition between the warm-season grass big bluestem *Schizachyrium scoparium* and the cool-season grass Canada wild rye *Elymus canadensis*, the presence of mycorrhizal fungi promoted the growth of the obligatory mycotrophic big bluestem over the facultatively mycorrhizae-dependent Canada wild rye. The degree of colonization that a species experiences can be correlated with the relative abundance of that species within the plant community. Another experiment by Hartnett and Wilson (1999) demonstrated the greater dependence of C₄ grasses on AMF compared to that of C₃ grasses. During their experiment they routinely applied the fungicide methyl bromide on an established prairie community for a period of five years. This reduced mycorrhizal colonization by 25% compared to the control which received no fungicide. This was enough to increase the presence

and diversity of the C₃ grasses while simultaneously decreasing the presence of the C₄ grasses.

When it comes to the restoration of tallgrass prairie ecosystems, little is known about the role of AMF. A prairie reconstruction in Minnesota using mycorrhizal inoculum exhibited an increase in percentage of ground covered by native grasses after 15 months of growth (Smith et al. 1998). Contrary to this finding, a follow-up study at a roadside prairie, also in Minnesota, showed that there was no increase in the percentage of native vegetation compared to the control (White et al. 2008). The study site in White et al. (2008) was high in phosphorus which likely negated the benefits associated with fungal colonization. There had been a previous attempt at reconstruction on this site resulting in native prairie plants being present for at least 7 years prior to their experiment. This likely caused the natural recolonization of AMF and would explain why the percentage of root colonization was equal in the control and inoculated plots after only 27 months after planting.

More studies need to be conducted to determine what affects mycorrhizal colonization has on restorations and reconstructions of tallgrass prairie ecosystem. A study by Zettler et al. (2001) found that the combination of cold stratification in conjunction to the presence of five species-specific AMF allowed for the germination and transplantation of the federally threatened eastern prairie fringed orchid (*Platanthera*

leucophaea). These findings demonstrate the potentially important roles that mycorrhizae can play in restoration efforts. However, more studies are needed to determine what effects mycorrhizal colonization has on tallgrass prairie restorations and reconstructions.

There is minimal information available in the literature regarding the effects of micronutrients in tallgrass prairie ecosystems. There is even less that specifically discusses the use of micronutrients in prairie restorations. Rothrock and Squiers (2003) studied the affects of annual applications of micronutrients in a prairie reconstruction in Indiana. Their experiment partially utilized land previously in row crop rotations. They found no consistent advantages to percent prairie species cover or prairie species density. However, they used only three micronutrients (B, Mn, and Zn) and surface applied the micronutrients instead of using a seed treatment.

Farooq et. al (2012) used seed priming to test the effects of individual micronutrients on the germination of common food crops and found that the micronutrients zinc, molybdenum, manganese, copper, and cobalt increased germination rates. Seed priming is a process where the seeds are partially hydrated in a nutrient solution and then dried out prior to germination. Babeva et. al (1999) found that seed priming with zinc sulfate increased germination of the prairie species *Echinacea purpurea* in both greenhouse and field settings.

My study was designed to test the effects of mycorrhizal inoculation and micronutrient seed treatments on the vegetative establishment of species used in a typical tallgrass prairie reconstruction at a site in Black Hawk County, Iowa. How the mycorrhizae and micronutrients effect seedling establishment and second year growth was examined comparing the functional plant groups of warm-season grasses, cool-season grasses, legumes, non-legume forbs. The effect of the treatments on weeds was also examined. The outcomes from this study will determine if any of these treatments significantly improve the success of a prairie reconstruction and if they are economically feasible.

CHAPTER 2

MATERIALS AND METHODS

Site Description

This experiment was designed to examine the effects of mycorrhizal inoculant and powdered micronutrient seed treatment on the early establishment of native vegetation during a tallgrass prairie reconstruction. The four treatments for this experiment were control, mycorrhizal inoculant, micronutrient seed treatment, and a combination of mycorrhizal inoculant and micronutrient seed treatment.

The research site selected for this experiment was located in a former agricultural field in the northwestern portion of the Cedar River Natural Resource Area (CRNA) in southeastern Black Hawk County, Iowa (42° 23' 28" N and 92° 13' 39" W). The CRNA is in the Cedar River flood plain, and this experiment was conducted on approximately 1 ha of 585 Spillville-Coland complex, a silty clay loam soil with 0-2% slope that is occasionally flooded.

Prior to this experiment, this site had been in long-term agricultural production with a corn and soybean crop rotation. During the final year of crop rotation the site was planted with soybeans (*Glycine max*) and was sprayed with glyphosate for weed suppression. The beans

were harvested in the fall of 2008 and the plots were laid out following harvest.

This experiment utilized a split-block design with each treatment being replicated three times in each block. A split-block design was used since there appeared to be slight differences in drainage between the eastern and western portions of the site. The east block was laid out with three rows of four plots each, while the west block had two rows of four plots, a row of two plots, and two rows with only one plot (Fig. 1). This created a stair step appearance to the western block and the plots were arranged in this manner to ensure that all plots remained in the same soil type while maximizing plot size (Fig. 2).

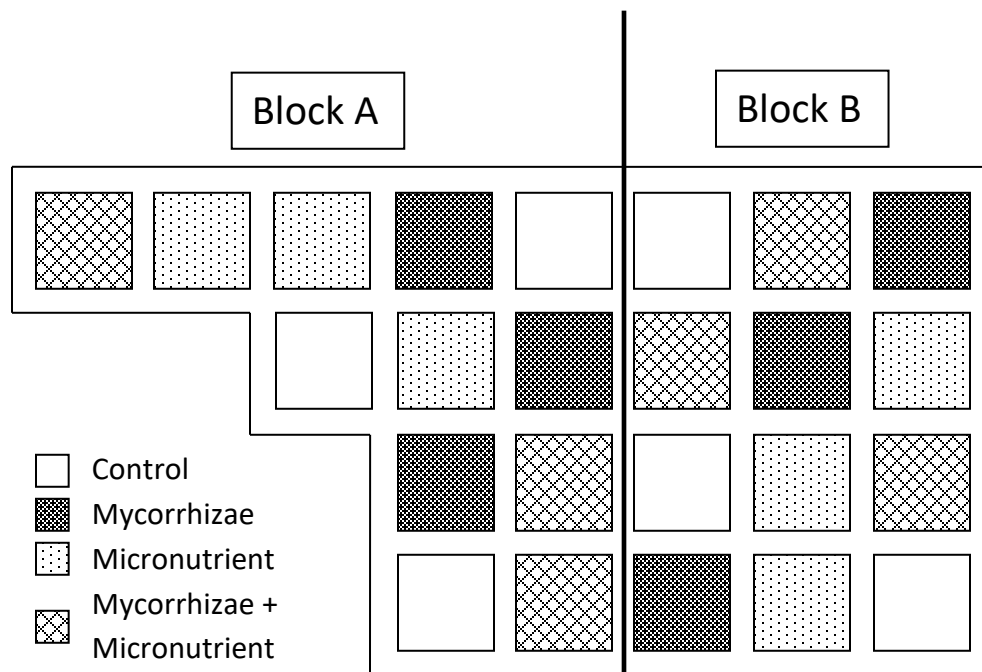


Figure 1. Plot and treatment map



Figure 2. Soil type aerial photo

All plots measured 17.5 m × 17.5 m with a 3 m buffer between each plot. The buffers not only marked plot boundaries but also prevented the underground spread of mycorrhizae between inoculated and non-inoculated plots. The buffers were planted with a cool-season pasture mix to provide ground cover and stabilize the soil. They were mowed to maintain a height of 6-10 cm throughout the experiment to prevent the non-native species from setting seed and invading the native vegetation.

Table 1. Soil nutrient analysis

	585 Spillville-Coland	
	Mean	SE
SOC*, g kg ⁻¹	25.1	0.88
TN†, g kg ⁻¹	2.29	0.08
pH	6.64	0.07
BD‡, g cm ⁻³	1.60	0.02
P, mg kg ⁻¹	85.4	4.93
K, mg kg ⁻¹	154.9	5.61
Ca, mg kg ⁻¹	3743	93.61
Mg, mg kg ⁻¹	599.7	16.00
S, mg kg ⁻¹	71.3	1.65
B, mg kg ⁻¹	1.10	0.05
Cu, mg kg ⁻¹	20.4	1.55
Fe mg kg ⁻¹	209.8	7.98
Mn, mg kg ⁻¹	138.8	6.09
Zn, mg kg ⁻¹	9.22	0.53

* Soil organic C content

† Total soil N

‡ Bulk density

The soil in the study area was tested for macro and micronutrients (Table 1) content as part of another experiment that was taking place at the same research site (Myers et. al 2015). They found this soil type to contain higher levels of soil nutrients when compared to other soil types found at the research site.

Table 2. Seed mix and seeding rate

Grasses		Phenology	Seeds/m²
Big Bluestem	<i>Andropogon gerardii</i>	WSG	86.11
Side-oats Grama	<i>Bouteloua curtipendula</i>	WSG	43.06
Prairie Brome	<i>Bromus kalmii</i>	CSG	21.53
Canada Wildrye	<i>Elymus canadensis</i>	CSG	21.53
Switchgrass	<i>Panicum virgatum</i>	WSG	86.11
Little Bluestem	<i>Schizachyrium scoparium</i>	WSG	53.82
Indian Grass	<i>Sorghastrum nutans</i>	WSG	53.82
Tall Dropseed	<i>Sporobolus asper</i>	WSG	32.29
		TOTAL (grass)	398.26
Forbs			
Leadplant	<i>Amorpha canescens</i>	Legume	10.76
Thimbleweed	<i>Anemone cylindrica</i>	Forb	2.69
Prairie Sage	<i>Artemisia ludoviciana</i>	Forb	21.53
Butterfly Milkweed	<i>Asclepias tuberosa</i>	Forb	5.38
New England Aster	<i>Aster novae-angliae</i>	Forb	10.76
Milk Vetch	<i>Astragalus canadensis</i>	Legume	32.29
White Wild Indigo	<i>Baptisia leucantha</i>	Legume	2.69
Partridge Pea	<i>Cassia fasciculata</i>	Legume	21.53
Prairie Coreopsis	<i>Coreopsis palmata</i>	Forb	2.69
Purple Prairie Clover	<i>Dalea purpurea</i>	Legume	32.29
Showy Tick Trefoil	<i>Desmodium canadense</i>	Legume	10.76
Pale Purple Coneflower	<i>Echinacea pallida</i>	Forb	10.76
Rattlesnake Master	<i>Eryngium yuccifolium</i>	Forb	5.38
Ox-eye Sunflower	<i>Heliopsis helianthoides</i>	Forb	10.76
False Boneset	<i>Kuhnia eupatoriodes</i>	Forb	10.76
Round-Headed Bush Clover	<i>Lespedeza capitata</i>	Legume	5.38
Rough Blazingstar	<i>Liatris aspera</i>	Forb	10.76
Wild Bergamot	<i>Monarda fistulosa</i>	Forb	21.53
Wild Quinine	<i>Parthenium integrifolium</i>	Forb	5.38
Foxglove Beardtongue	<i>Penstemon digitalis</i>	Forb	15.07
Prairie Phlox	<i>Phlox pilosa</i>	Forb	2.69
Common Mt. Mint	<i>Pycnanthemum virginianum</i>	Forb	32.29
Yellow Coneflower	<i>Ratibida pinnata</i>	Forb	32.29
Black-eyed Susan	<i>Rudbeckia hirta</i>	Forb	21.53
Compass Plant	<i>Silphium laciniatum</i>	Forb	1.08
Stiff Goldenrod	<i>Solidago rigida</i>	Forb	32.29
Prairie Spiderwort	<i>Tradescantia bracteata</i>	Forb	5.38
Golden Alexanders	<i>Zizia aurea</i>	Forb	21.53
		TOTAL (forb)	398.26
		TOTAL	796.53

Seed Mix and Amendments

The design of the seed mix used for this experiment was based on the location, soil type, and hydrology of the site. The seed mixture was comprised of 36 species including 8 grass species (6 warm-season and 2 cool-season) and 28 forb species (21 non-legumes and 7 legumes) (Table 2). Seeding rates were determined using seeds per m² for each species. The mix used a 1:1 ratio of grasses to forbs and each were planted at a rate of 398.26 seeds per m².

The seed for each species was weighed out individually for each of the 24 plots to ensure that all plots received an equal amount of seed for each species. Pure live seed (PLS) weights were used instead of bulk seed weight. Zone 2, source-identified yellow tag certified seed was purchased for the species of which it was available. When yellow tag certified seed was not available, uncertified seed with the nearest local origin was used.

Once the seed was weighed for each plot, mycorrhizal inoculant and micronutrient seed powder was added to the seed mix for the designated plots. MycoApply® Endo was the mycorrhizal inoculum used and is produced by Mycorrhizal Applications, Inc. It contains 27,216 propagules/kg of the endomycorrhizal species *Glomus intraradices*, *G. mosseae*, *G. aggregatum*, and *G. etunicatum*. The inoculum was applied

at a rate of 22.4 kg/ha, or 0.685 kg/plot, as per the manufacturers recommendation for restorations.

The micronutrient powder used was Nutriplant® SD 0-0-0 which is distributed by Access Business Group International LLC. The contents of the micronutrient powder used is below (Table 2). It was applied at a rate of 99.2 g per 45.4 kg of seed as per the manufacturer's recommendation for barley, oats, rice, rye, and wheat. The nutrients used were derived from calcium sulfate, magnesium oxide, magnesium sulfate, cobalt nitrate, copper sulfate, ferrous sulfate, manganese sulfate, ammonium molybdate, and zinc sulfate.

Table 3. Chemical analysis of micronutrient seed powder

Micronutrient	Percentage by weight	% Water Soluble
Calcium (Ca)	4.0%	-
Magnesium (Mg)	2.0%	1.0%
Sulfur (S)	4.0%	4.0%
Cobalt (Co)	0.001%	-
Copper (Cu)	0.075%	0.075%
Iron (Fe)	1.0%	1.0%
Manganese (Mn)	0.25%	0.25%
Molybdenum (Mo)	0.0005%	-
Zinc (Zn)	1.0%	1.0%

Planting Method and Plot Management

The prairie research plots were planted May 17th and 18th, 2009. All plots were planted using a 4 foot Truax no-till seed drill fitted with a special seeding tube attachment to ensure that all seed was sown and not caught up in the drill. The front tillage discs were removed from the drill to ensure that seeds were not placed too deep in the soil. The drill was cleaned between treatments to ensure there was no cross-contamination among treatments. Crushed clay chips were added to the seed mixture for the plots that did not contain the mycorrhizal inoculant. The purpose of this was to equalize the flow rates of seed through the drill for all treatments since the granular mycorrhizal inoculant increased the rate of seed flow (Fig. 3).



Figure 3. Seed drill modification for planting small areas

Establishment mowing was performed on June 26th of the first growing season at a height of approximately 10 cm. There was little weed growth and the native vegetation had excellent establishment during the first growing season. Therefore, a second establishment mowing was not needed during the first growing season.

Sampling and Data Analysis

Year 1 vegetation sampling was conducted in late July 2009. Data was collected by counting the number of seedlings for each native and weed species within 15 randomly placed 0.10 m² quadrats. Biomass data was collected by randomly selecting 5 of the 15 quadrats to be clipped at ground level and sorted into native grass, native forbs, and weed species. The plant biomass was then dried to a constant mass and weighed.

Shortly after vegetation sampling was complete it was observed that forbs appeared to be flowering at a higher rate in the treatment plots compared to the control plots. To quantify this, the number of flowering plants was recorded in each plot for each species while walking four evenly spaced transects that ran north and south. This effectively covered the entirety of each plot. Plants with multiple buds at anthesis were counted the same as plants with only one. For the purpose of this

experiment anthesis was considered to have been reached if there was any visible color to the corolla or ray flowers for the members of the aster family. The flowering data was treated as an indicator of comparative plant maturity.

By the second growing season it was not possible to count individual seedlings or plants due to excessive growth and tillering. For the second sampling period, data was collected by estimating the percentage of basal coverage of each species found within 10 randomly placed 0.10 m² quadrats, and the number of biomass clippings was increased from 5 quadrats to 7 quadrats. The number of quadrats sampled was reduced due to increased uniformity in the vegetation, and the sample size for the biomass clippings were increased to account for variability and increase statistical power.

Data for both years were analyzed using a 2-way ANOVA that used treatment as a fixed effect and block as a random factor. A Tukey's protected test for pairwise comparison was used to compare means among treatments. The significance threshold for the data analyzed was $\alpha \leq 0.05$ and results are referred to as marginally significant when $0.10 \leq \alpha > 0.05$. Block 1 and Block 2 data were compared for any block-by-treatment interactions. If no interactions were discovered Block 1 and Block 2 data were combined for data analysis. Year 1 and Year 2

data were not compared to each other since two different methods of data collection were used.

At the end of the second year of establishment soil cores were collected in order to gather root material to be evaluated for mycorrhizal colonization. Plots were randomly sampled and 15 soil cores were taken from each plot using a 22 mm diameter soil probe at a depth of 25 cm. The soil cores were then combined and washed to separate soil and inert material from root tissue. The root tissue was then cut into 1 cm sections with a razor blade.

The root tissue was then placed in tissue cassettes and cleared in a 10% KOH solution heated to 90° C for two hours. The samples were then thoroughly rinsed in tap water to remove the KOH solution. Next, the root tissue was dyed with trypan blue in a 0.05% weight to volume solution with glycerol for 6 days. This process stains the mycorrhizae a deep blue while having little to no color effects on the cleared root tissue.

After the staining process was complete, 0.15 g of the 1 cm long tissue sections was randomly spread out on a 9 cm diameter petri dish with 12.7 mm x 12.7 mm gridlines inscribed on the bottom. The petri dish was viewed under a dissecting microscope and each place where the root tissue intersected a horizontal or vertical gridline was counted. It was also recorded if the section of root at the intersection point was colonized by mycorrhizae. The number of colonized intersections was

divided by the number of total intersections to determine the percent colonization. There were 5 subsamples tested for each plot and the average number of intersections recorded was 312 per plot with a high of 485 and a low of 265.

CHAPTER 3

RESULTS

Year 1 Results

Table 4. Seedling count means

BLK	TRMN	TTL GRASS	WSG	CSG	TTL FORB	LEGUME	NON-LEGUME	TTL NATIVE	WEED
1	1	58.00	50.00	8.00	27.33	20.00	7.33	85.33	41.33
1	1	47.33	38.00	9.33	22.67	12.00	10.67	70.00	9.33
1	1	39.33	35.33	4.00	41.33	18.67	22.67	80.67	20.00
2	1	49.33	43.33	6.00	16.00	8.67	7.33	65.33	44.67
2	1	45.33	36.00	9.33	30.00	9.33	20.67	75.33	57.33
2	1	40.67	35.33	5.33	20.67	8.67	12.00	61.33	65.33
1	2	70.00	63.33	6.67	36.67	15.33	21.33	106.67	38.67
1	2	78.67	64.67	14.00	57.33	30.00	27.33	136.00	19.33
1	2	62.67	52.00	10.67	52.00	24.00	28.00	114.67	23.33
2	2	60.00	54.00	6.00	19.33	10.67	8.67	79.33	123.33
2	2	56.00	50.00	6.00	40.67	20.67	20.00	96.67	34.00
2	2	78.67	70.67	8.00	67.33	38.00	29.33	146.00	12.67
1	3	62.00	55.33	6.67	28.00	12.00	16.00	90.00	66.00
1	3	63.33	52.67	10.67	42.00	24.67	17.33	105.33	14.00
1	3	64.00	57.33	6.67	41.33	16.67	24.67	105.33	20.67
2	3	52.00	40.00	12.00	25.33	14.67	10.67	77.33	63.33
2	3	73.33	63.33	10.00	21.33	14.67	6.67	94.67	24.67
2	3	81.33	66.00	15.33	40.00	22.00	18.00	121.33	50.00
1	4	52.67	42.67	10.00	35.33	11.33	24.00	88.00	42.67
1	4	65.33	53.33	12.00	29.33	20.00	9.33	94.67	36.00
1	4	64.00	54.00	10.00	28.00	12.67	15.33	92.00	24.67
2	4	77.33	67.33	10.00	56.67	15.33	41.33	134.00	56.00
2	4	62.00	54.00	8.00	47.33	29.33	18.00	109.33	44.00
2	4	48.67	43.33	5.33	43.33	24.00	19.33	92.00	36.00

In all tables and figures in the results section Treatment 1 is the control, Treatment 2 is mycorrhizal inoculant, Treatment 3 is micronutrient seed treatment, and Treatment 4 is the combination of

Treatments 3 and 4. WSG and CSG equate to warm-season grass and cool-season grass respectively. There were no block by treatment interactions throughout the entirety of the study. Only Year 1 weed seedlings, Year 2 grass biomass, and Year 2 total native biomass did the results vary between Block A and Block B.

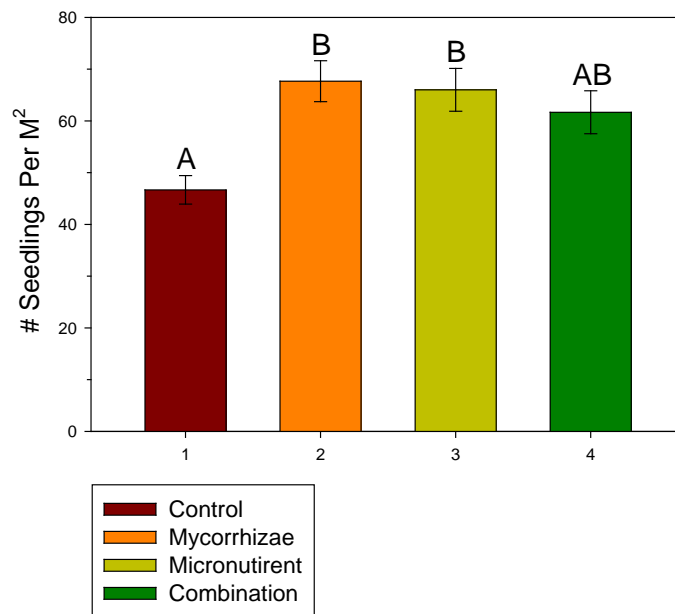


Figure 4. Total grass seedling count

There was a 31.1% ($p=0.011$) and 29.3% ($p=0.019$) increase in native grass seedlings in the mycorrhizae and micronutrient treatments respectively over the control. There was an increase in the combination plots, however it was not significant at $p=0.083$.

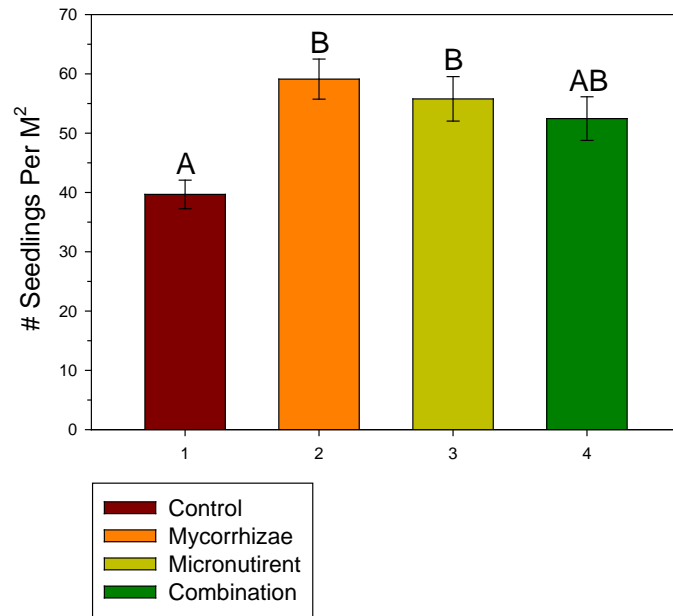


Figure 5. Warm-season grass seedling count

There were significant differences in treatment ($p=0.009$) in the average number of warm-season grass seedlings. The mycorrhizae and the micronutrient treatments were significantly higher ($p=0.008$ and $p=0.031$) than the number of WSG seedlings found in the control. The combination treatment was marginally significant ($p=0.083$) in having greater numbers of WSG seedlings than the control, and it was also statistically similar ($p=0.584$ and $p=0.916$) to both the mycorrhizae and micronutrient treatments.

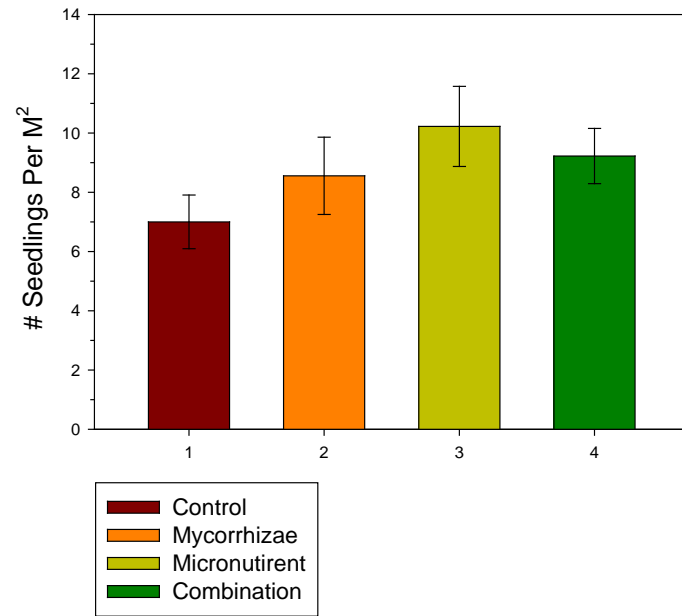


Figure 6. Cool-season grass seedling count

No statistical difference ($p=0.172$) in treatment in the number of native cool-season grasses that were present in Year 1.

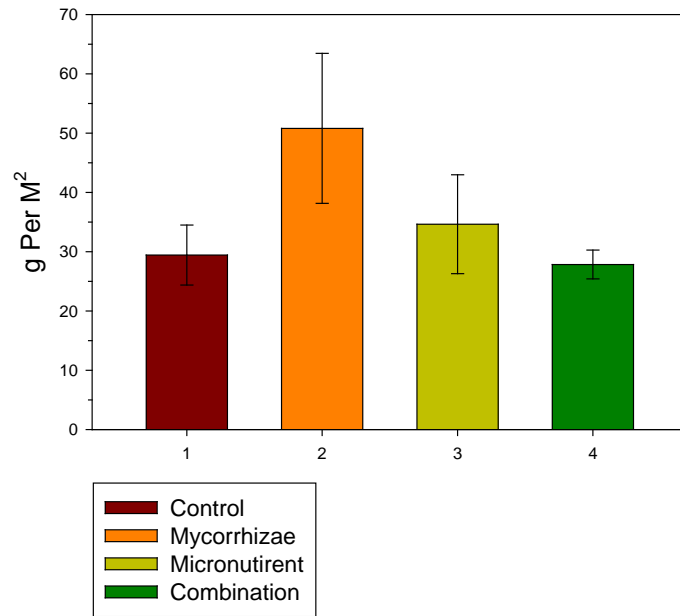


Figure 7. Year 1 total grass biomass

No significant differences ($p=0.223$) in biomass harvesting among any of the treatments. There was a great deal of variability with a standard error of 12.64 g/M^2 which may have contributed to the lack of statistical significance.

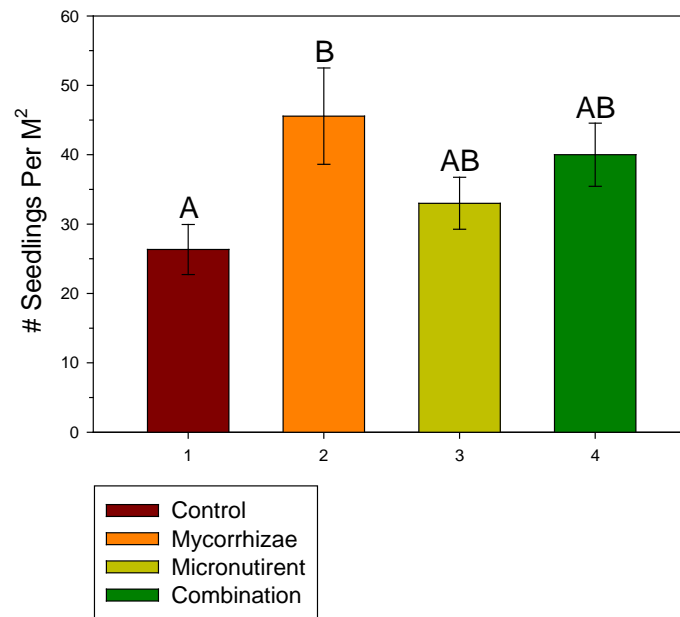


Figure 8. Total forb seedling count

There were 42.2% ($p=0.047$) more native forb seedlings in the mycorrhizae plots than in the control, and no significant differences between the micronutrient treatment ($p=0.750$) or the combination treatment ($p=0.209$).

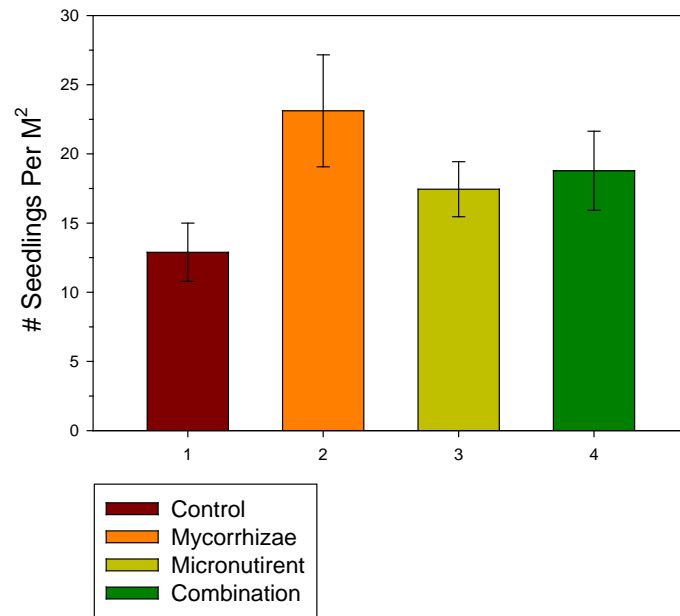


Figure 9. Legume seedling count

No statistical differences ($p=0.133$) were seen in legume seedlings by treatment. However, the number of legume seedlings present was marginally significant ($p=0.095$) in the mycorrhizal treatment over the control.

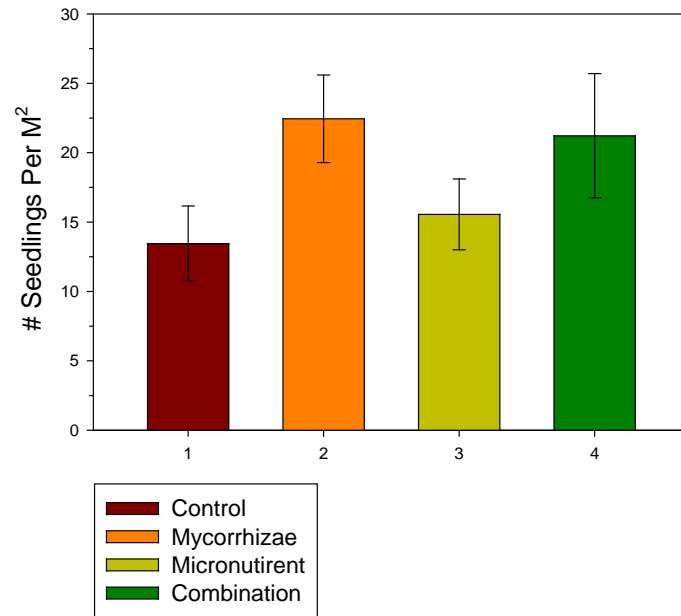


Figure 10. Non-legume forb seedling count

The average number of non-legume forb seedlings did not vary significantly by treatment ($p=0.193$).

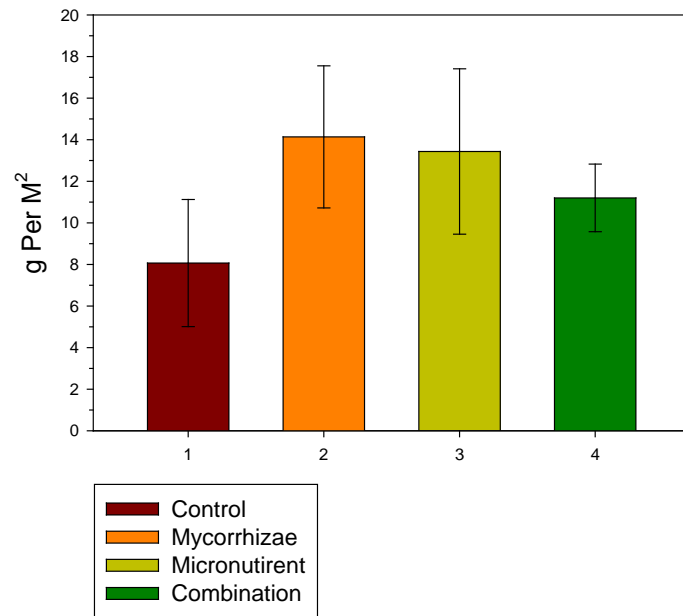


Figure 11. Year 1 biomass of forbs

No significant differences ($p=0.523$) were seen in forb biomass during the first growing season. There was a high amount of variability within each treat which is similar to the other Year 1 biomass calculations.

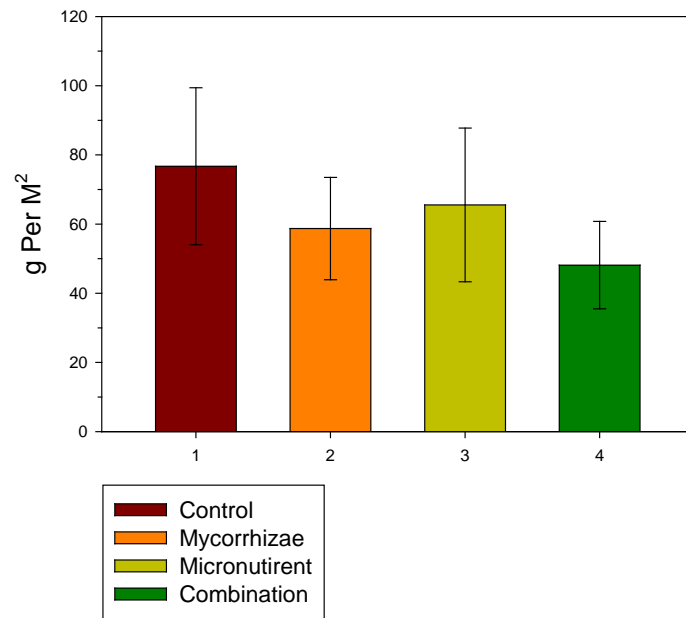


Figure 12. Year 1 weed biomass

There were no significant differences among treatments ($p=0.713$) in the biomass of weed specie.

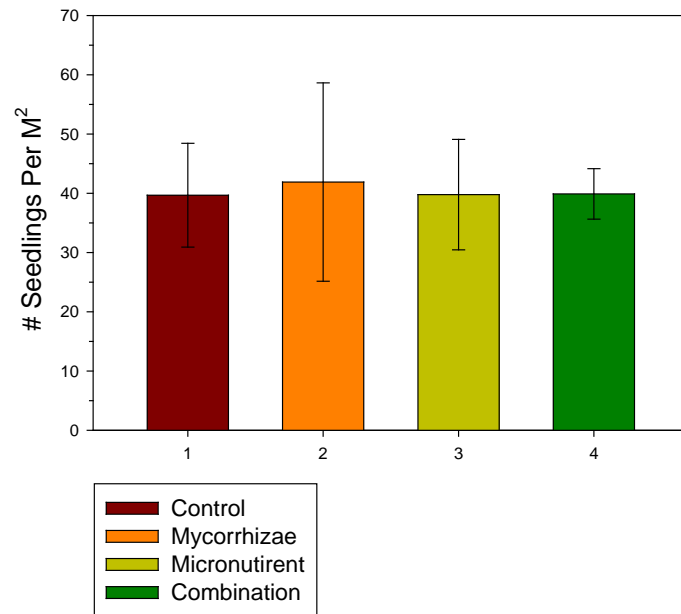


Figure 13. Weed seedling count

There was no treatment effect on average number of weed seedlings ($p=0.994$). There were significant differences by block ($p=0.049$).

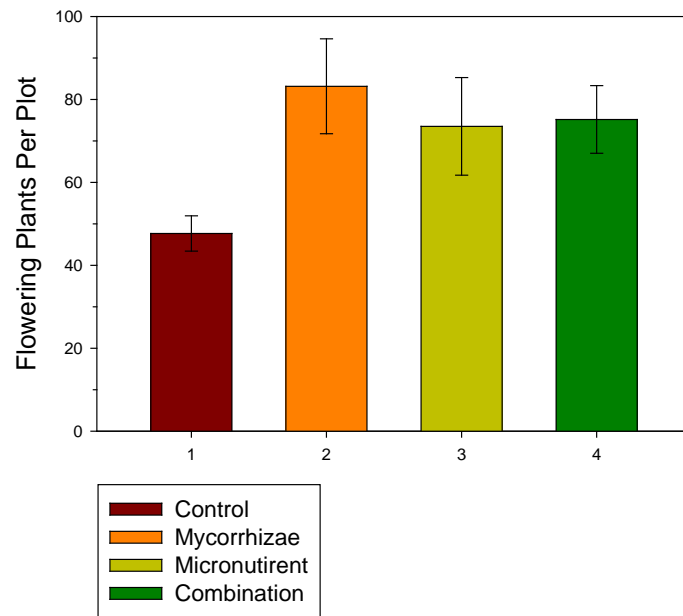


Figure 14. Total flowering forbs

There was a marginally significant increase in flowering of all forb species across all three treatments ($p=0.104$).

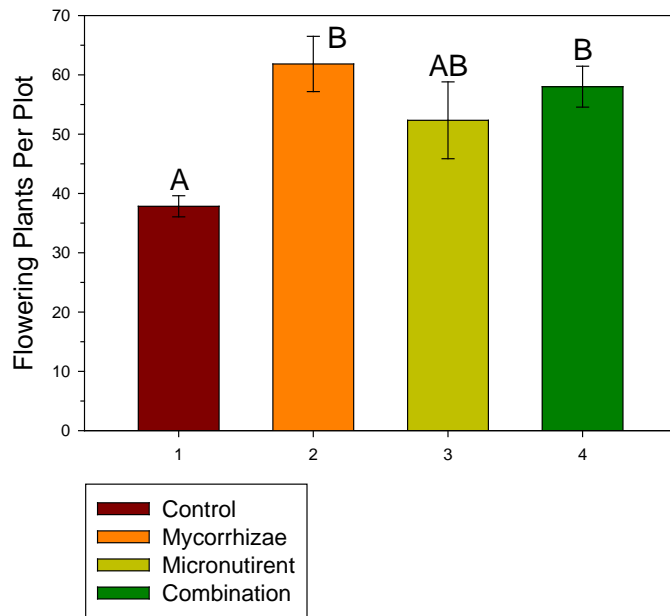


Figure 15. Flowering *C. fasciculata* count

The flowering time data revealed that there was 38.8% ($p=0.009$) and 34.8% ($p=0.029$) increase in partridge pea (*Chamaecrista fasciculata*) flowering at the time of sampling (08/06/2009) in the mycorrhizae and combination treatment plots over the control plots.

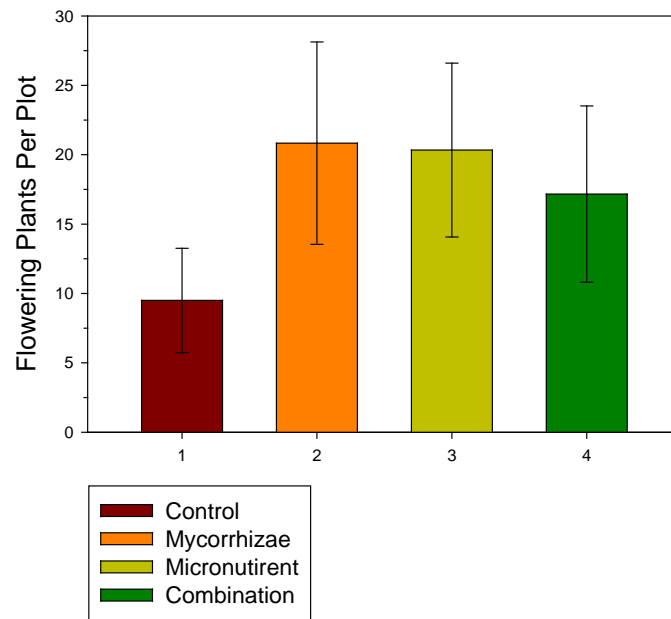


Figure 16. Flowering *R. hirta* count

There were no significant differences in black-eyed susan (*Rudeckia hirta*) flowering by treatment ($p=0.584$).

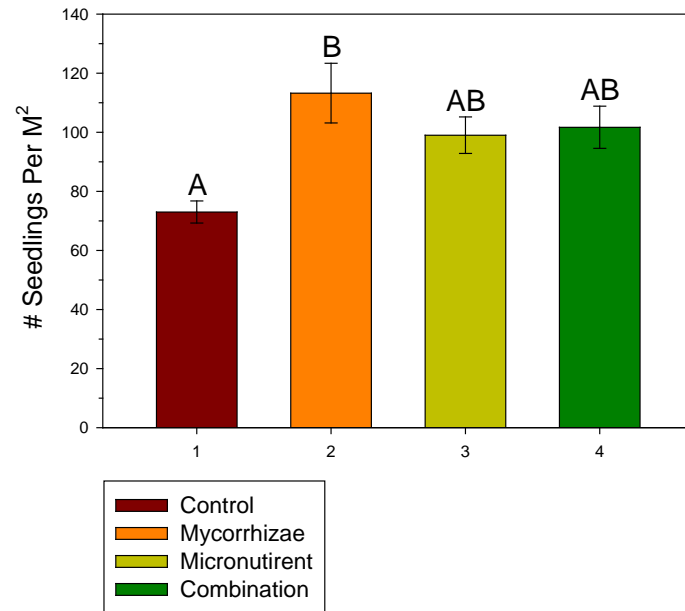


Figure 17. Total native seedling count

An increase of 35.5% ($p=0.006$) in total native seedlings in the mycorrhizae plots over the control plots. The micronutrient and combination treatments showed an increase in total native seedlings but fell outside of the confidence interval with $p=0.096$ and $p=0.059$ respectively.

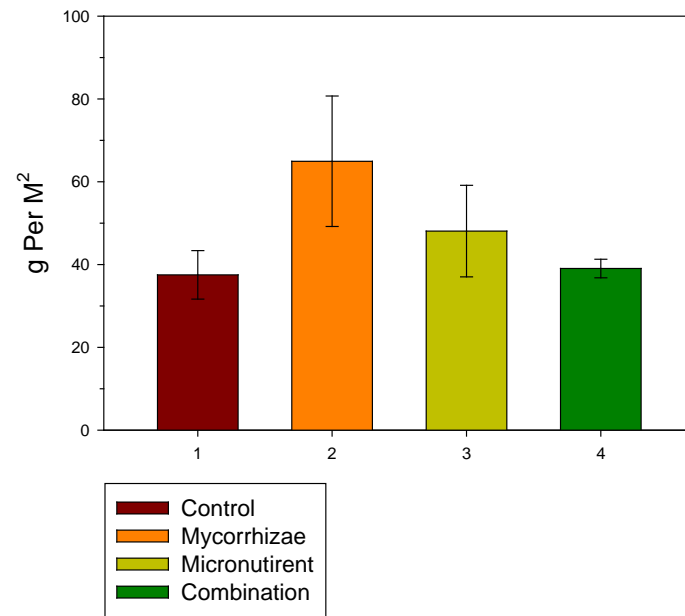


Figure 18. Year 1 total native biomass

The biomass of planted native species in the mycorrhizae treatment compared to the control demonstrated no significant treatment differences ($p=0.248$).

Table 5. Mean biomass (g) of native plant groups

TRMNT	TOTAL GRASS	TOTAL FORB	TOTAL NATIVE	WEEDS
1	29.433	8.067	37.500	76.700
2	50.800	14.133	64.933	58.700
3	34.633	13.433	48.067	65.533
4	27.833	11.200	39.033	48.133

There was no statistical difference between treatments for any of the plant types sampled.

Year 2 Results

Table 6. Average percent basal coverage by native plant group

BLK	TRMN	TTL GRASS	WSG	CSG	TTL FORB	LEGUME	NON-LEGUME	TTL NATIVE	WEED
1	1	0.448	0.243	0.206	0.093	0.033	0.059	0.541	0.070
1	1	0.402	0.358	0.044	0.127	0.070	0.058	0.529	0.042
1	1	0.329	0.220	0.109	0.069	0.021	0.048	0.398	0.110
2	1	0.331	0.229	0.102	0.073	0.031	0.042	0.404	0.031
2	1	0.354	0.236	0.118	0.101	0.045	0.056	0.455	0.081
2	1	0.426	0.268	0.157	0.062	0.028	0.034	0.487	0.092
1	2	0.676	0.516	0.160	0.273	0.083	0.190	0.949	0.018
1	2	0.732	0.311	0.421	0.225	0.057	0.168	0.957	0.010
1	2	0.674	0.441	0.233	0.309	0.048	0.261	0.983	0.034
2	2	0.945	0.649	0.296	0.270	0.074	0.197	1.215	0.090
2	2	0.638	0.391	0.247	0.246	0.094	0.153	0.884	0.027
2	2	0.488	0.373	0.115	0.349	0.063	0.286	0.837	0.031
1	3	0.549	0.411	0.139	0.151	0.031	0.120	0.700	0.091
1	3	0.563	0.301	0.262	0.243	0.078	0.165	0.805	0.010
1	3	0.559	0.370	0.190	0.238	0.060	0.178	0.797	0.023
2	3	0.477	0.307	0.170	0.183	0.065	0.118	0.661	0.055
2	3	0.665	0.464	0.200	0.221	0.112	0.109	0.885	0.020
2	3	0.784	0.276	0.508	0.179	0.065	0.114	0.963	0.040
1	4	0.650	0.392	0.258	0.147	0.021	0.126	0.797	0.041
1	4	0.527	0.336	0.192	0.376	0.071	0.306	0.904	0.026
1	4	0.695	0.480	0.215	0.242	0.045	0.197	0.937	0.021
2	4	0.555	0.352	0.203	0.423	0.032	0.391	0.978	0.054
2	4	0.449	0.304	0.145	0.268	0.069	0.200	0.718	0.035
2	4	0.549	0.361	0.188	0.240	0.070	0.169	0.789	0.021

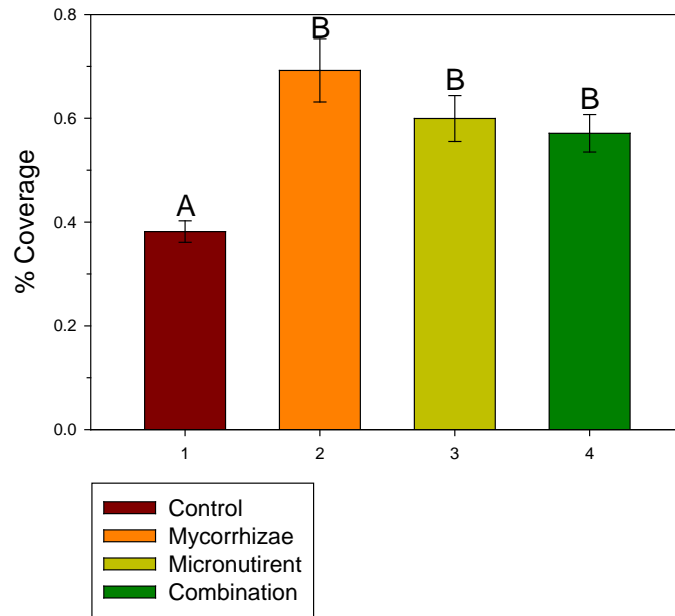


Figure 19. Basal coverage of total native grasses

The differences in basal coverage of native grasses was highly significant ($p=0.001$) in all three treatments when compared to the control. There was no differences between any of the amended treatments.

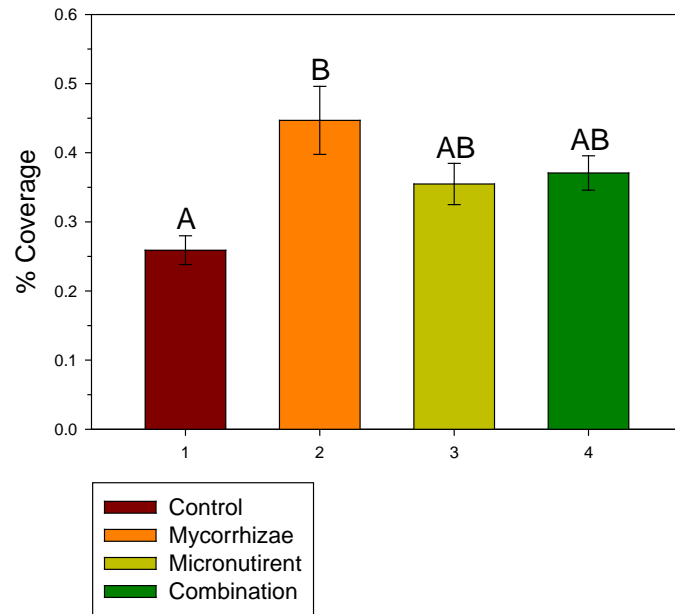


Figure 20. Basal coverage of warm-season grasses

Treatment differences in basal coverage of warm-season grasses were significant ($p=0.015$). The mycorrhizae treatment exhibited a significant increase ($p=0.008$) over the control while the micronutrient and combination treatments were not significantly different from the control ($p=0.260$ and 0.155).

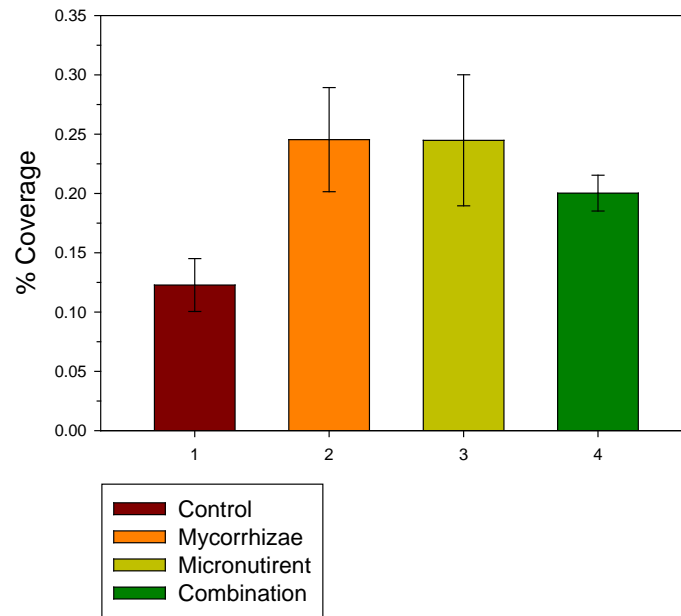


Figure 21. Basal coverage of cool-season grasses

The overall treatment effects on basal coverage of cool-season grasses were only marginally significant ($p=0.078$). When compared to the control, the mycorrhizae, micronutrient, and combination treatments had p-values of 0.097, 0.108, and 0.155 respectively.

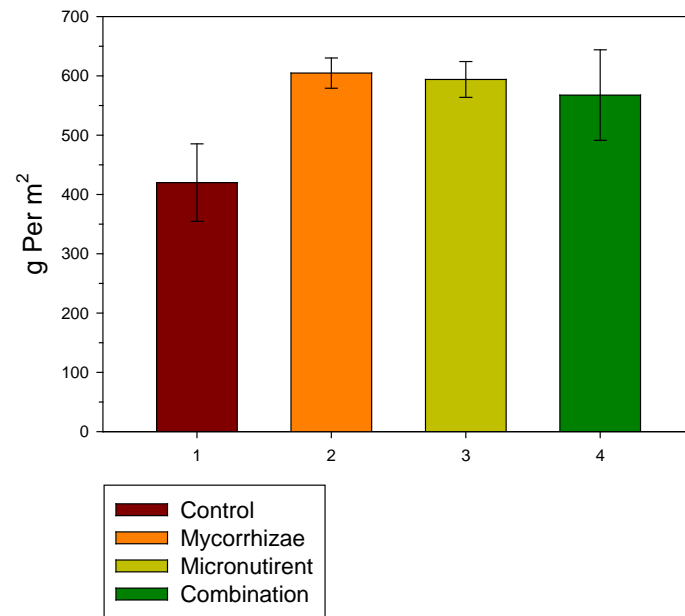


Figure 22. Year 2 native grass biomass

There was no significant differences ($p=0.155$) in the biomass of native grasses in Year 2. There was a block difference ($p=0.038$).

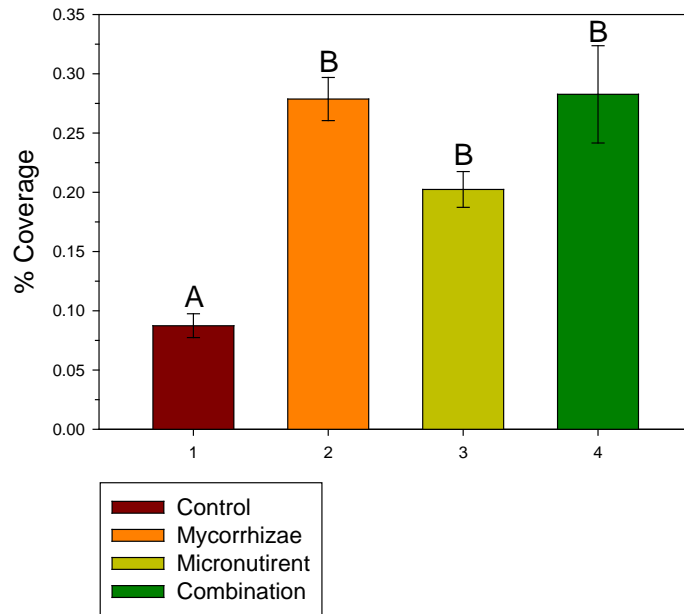


Figure 23. Basal coverage of total forbs

Significant treatment differences ($p=0.000$) were seen in the total basal coverage of native forb species. Despite an overall increase, neither treatments containing the mycorrhizal inoculant were significantly higher than the micronutrients ($p=0.201$ and $p=0.168$).

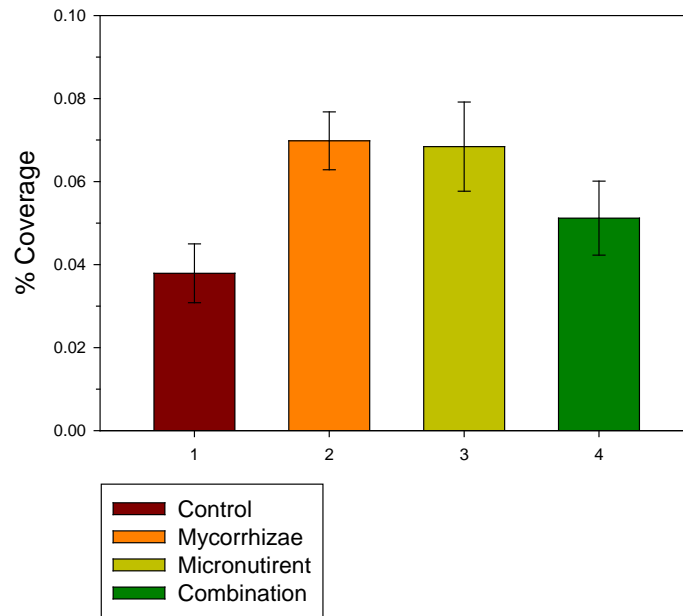


Figure 24. Basal coverage of legumes

Basal coverage of legumes experienced a marginally significant differences in Year 2 ($p=0.063$). No difference was seen between the mycorrhizae and the micronutrient treatments ($p=1.000$).

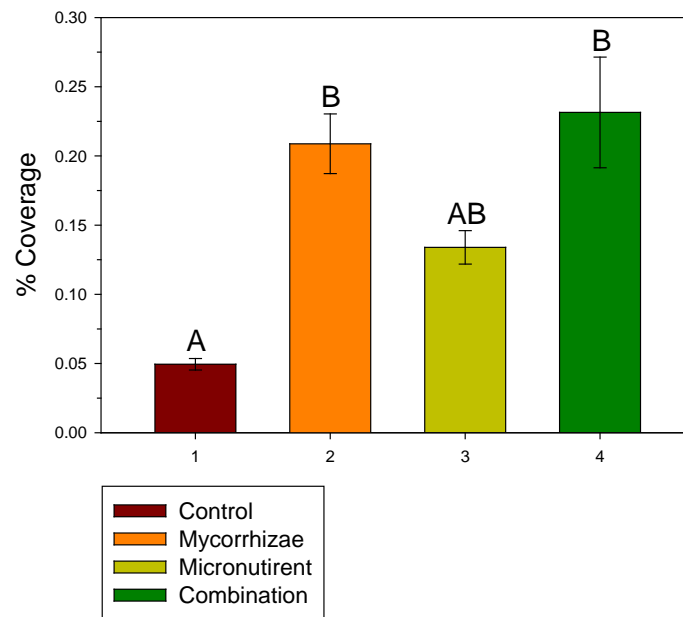


Figure 25. Basal coverage of non-legume forbs

There were strongly significant differences in the basal coverage of non-legume forbs of the mycorrhizal and combination treatments when compared to the control ($p=0.002$ and $p=0.001$). Although the micronutrient treatment showed increased basal coverage of non-legume forbs it was not significant ($p=0.125$). The combination treatment had a marginally significant increase in the percentage of basal coverage when compared to the micronutrient treatment ($p=0.065$).

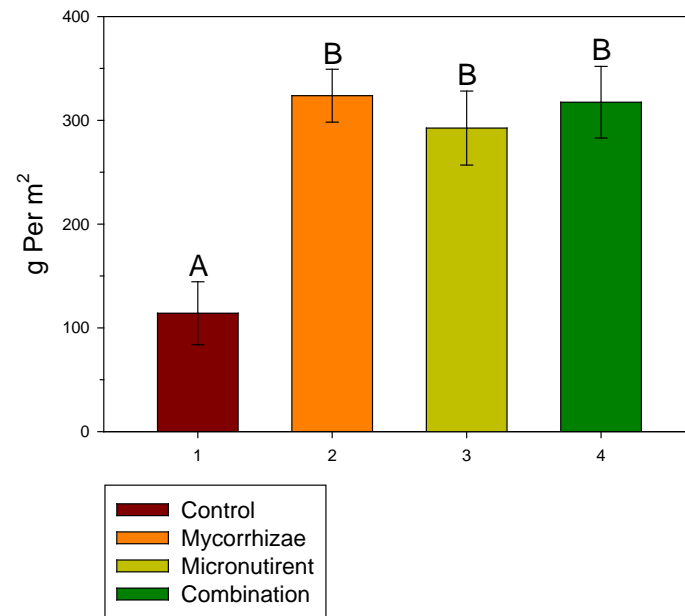


Figure 26. Year 2 forb biomass

There were strong treatment differences ($p=0.002$) in the biomass of native forbs in Year 2. No block ($p=0.971$) or block by treatment differences ($p=0.656$) were seen.

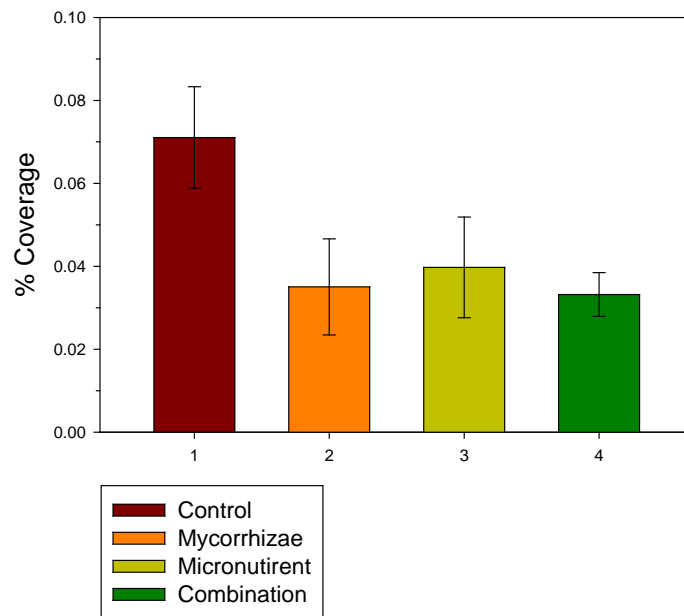


Figure 27. Basal coverage of weeds

The decrease in basal coverage of weed species across all treatments was only marginally significant ($p=0.102$).

Table 7. Year 2 biomass(g) by plant group

Treatment	Grass Biomass	Forb Biomass	Total Native Biomass	Weed Biomass
1.0	420.0	114.1	534.1	25.5
2.0	604.7	323.7	928.4	16.9
3.0	593.9	292.5	886.4	34.0
4.0	567.6	317.4	885.0	17.9

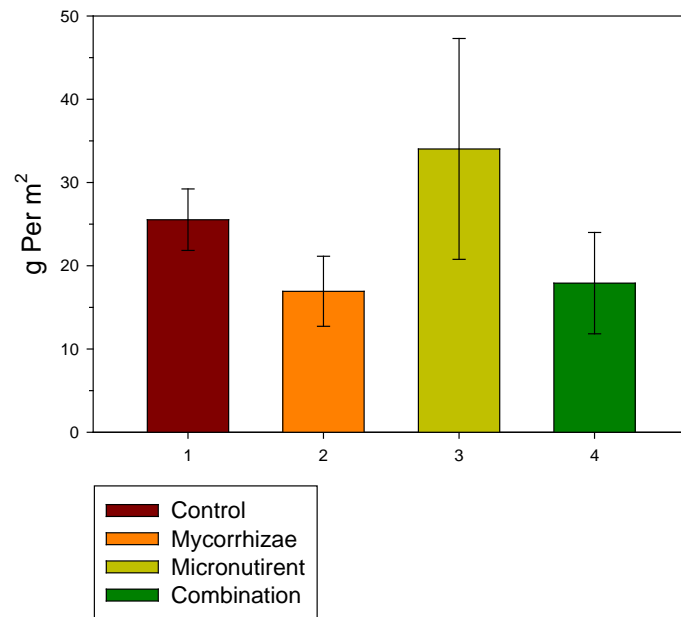


Figure 28. Year 2 weed biomass

There were no significant differences by treatment ($p=0.368$) of non-native species. Overall weed pressure was low in both blocks with an average dry mass of 25.58 g across all treatments compared to an average of 808.5 g for total natives. The high variability in standard errors is also a product of low weed pressure.

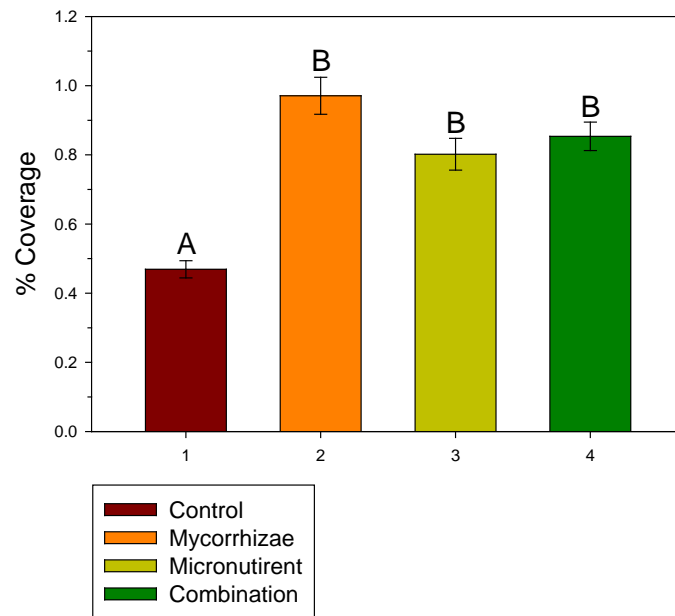


Figure 29. Basal coverage of total natives

Significant differences by treatment ($p=0.000$) were present across all treatments. The difference between mycorrhizal and micronutrient treatments was marginally significant ($p=0.083$).

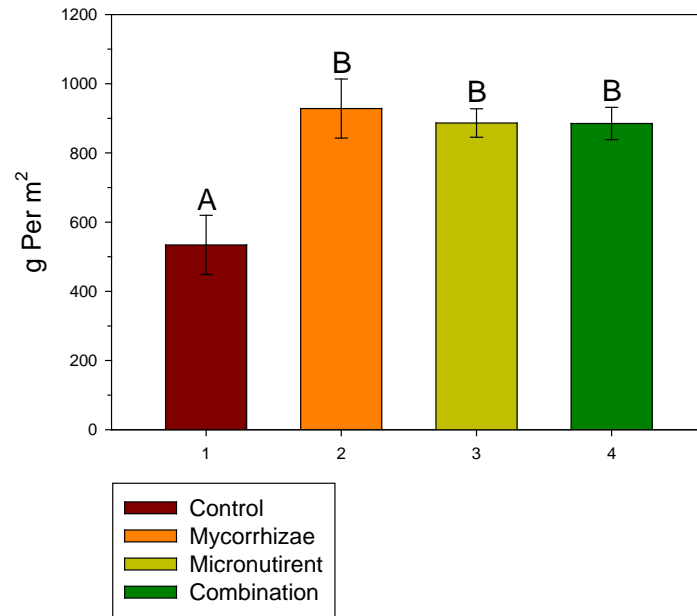


Figure 30. Year 2 total native biomass

There were significant differences ($p=0.001$) in total biomass yield of natives during Year 2 sampling across all treatments. There was also a significant difference by block ($p=0.049$).

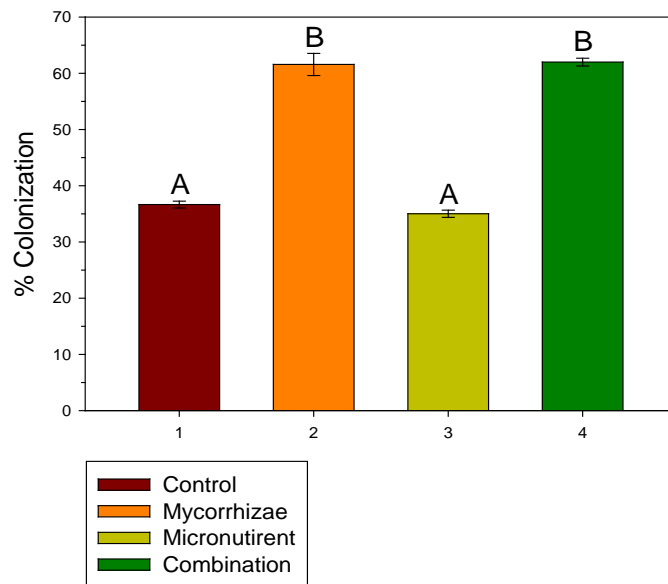


Figure 31. Mycorrhizal root colonization percentages

Root sampling revealed that there was a significant increase ($p=0.000$) in mycorrhizal fungal colonization in the treatments that had mycorrhizal inoculant added to the seed mixture over plots that did not receive inoculant. Inoculation resulted in 61.8% mycorrhizal root colonization versus 35.8% colonization in non-inoculated treatments.

Table 8. Mean mycorrhizal colonization percentages

Treatment	Block	Mean Colonization
1	1	36.32 %
1	2	36.96 %
2	1	58.60 %
2	2	64.52 %
3	1	34.65 %
3	2	35.38 %
4	1	61.44 %
4	2	62.52 %

Table 9. Plant response to mycorrhiza and micronutrient treatments

	Mycorrhizal	Micronutrient	Both
Avg. native seedling	+*	+**	+**
Avg. native grass seedlings	+*	+*	+**
Avg. warm-season grass seedlings	+*	+*	+**
Avg. cool-season grass seedlings	+	+	+
Biomass of native grasses Year 1	+	+	0
Avg. native forb seedlings	+*	+	+
Avg. legume seedlings	+**	+	+
Avg. non-legume forb seedlings	+	0	+
Biomass of forbs Year 1	+	+	+
Biomass of weeds Year 1	-	0	-
Avg. weed seedlings	0	0	0
Number of flowering natives on 8/6/09	+**	+	+
Number of flowering partridge pea 8/6/09	+*	+	+*
Flowering black-eyed Susan on 8/0/09	+	+	+
Biomass of total natives Year 1	+	+	0
Basal coverage of native grasses	+*	+*	+*
Basal coverage of warm-season grasses	+*	+	+
Basal coverage of cool-season grasses	+**	+**	+
Biomass of native grasses Year 2	+	+	+
Basal coverage of native forbs*	+*	+*	+*
Basal coverage of legume	+**	+**	+
Basal coverage of non-legume forbs*	+*	+	+*
Biomass of native forbs Year 2	+*	+*	+*
Basal coverage of weed species	-	-	-
Biomass of weeds Year 2	-	0	-
Basal coverage of planted natives	+*	+*	+*
Biomass of total natives Year 2	+*	+*	+*
Mycorrhizal root colonization	+*	0	+*

Increase = + decrease = - no difference = 0
 significant = * marginally significant = **

Interactions

There were no block x treatment interactions in this study. There were block effects seen in Year 1 weed seedlings where block A had a higher average of weed seedlings than block B. The most common weed found in Year 1 was Siberian elm saplings and there was a row of Siberian elm trees that ran adjacent to the eastern border of block A. There was also a block effect in total native biomass and grass biomass in Year 2. This was a result of block B being slightly lower in elevation and it did not drain water as well. During the spring of the second growing season there was about 2” of standing water on the most of block B for about one week resulting slower growth rates and the possible loss of a few individuals.

CHAPTER 4

DISCUSSION

High quality native seed, land cost, seeding equipment costs, and staff salaries make tallgrass prairie reconstruction a relatively expensive endeavor. The purpose of this experiment was to evaluate if either of the two seeding amendments or a combination of the two have the potential to improve the cost efficiency of a prairie reconstruction. It is important to remember when examining plant-fungi, plant-nutrient, and plant-fungi-nutrient interactions that the nature of the relationship becomes a sliding scale. This experiment examined plant-fungi-nutrient relationships where mycorrhizae and micronutrients were added as soil amendments. The biotic and abiotic properties of the experimental site also played a major role in determining the relationship between the three factors. Given the complex nature of these interactions these outcomes are representative of those interactions at the experiment site at a given point in time (Johnson et al. 1997).

All three treatments enhanced the establishment of the native plant community during the first two growing seasons. However, in most instances the addition of only mycorrhizal inoculant slightly outperformed other treatments. The results of adding micronutrients closely resembled those of the mycorrhizal inoculant's in most cases and

although the benefits were typically less than mycorrhizal inoculant it was usually not significantly different. The combination treatment of mycorrhizal inoculant and micronutrient also improved establishment of the native plant community in comparison to the control. However, adding both mycorrhizae and micronutrients did not yield a cumulative positive response when compared to the only mycorrhizal and only micronutrient treatments. The benefits of the combination treatments were typically less than the other treatments, but not usually significantly. This was quite possibly a result of the associated metabolic costs of the native plants sharing their resources with the fungal colonies and not receiving as much benefit in return. The presence of supplemental micronutrients means that micronutrients are no longer a limiting factor for growth and the benefits received from the mycorrhizae become diminished.

During Year 1 of establishment there were significant differences seen between the mycorrhizal treatment and the control in total native seedlings, native grass seedlings, warm-season grass seedlings, number of forb seedlings, and the number of partridge pea (*C. fasciculata*) that flowered during the first growing season. Additionally, there were marginally significant ($p < 0.134$) increases in legume seedlings, non-legume forb seedlings, and total flowering natives. There were no differences in cool-season grass seedlings, weed seedlings, or the number

of flowering black eyed Susan. Biomass results in Year 1 had large standard errors for all plant groups studied. This was a product of the highly variable nature in the first year of a prairie reconstruction and low sample size.

In the second year of the experiment basal coverage of total natives, native grasses, warm-season grasses, forbs, and non-legume forbs in the mycorrhizal treatment was significantly greater than the control. The only areas that did not show significant changes in basal coverage were cool-season grasses, legumes, and weed species although there were strong trends ($p < 0.103$) in all three categories. In addition, the biomass of total natives and total forbs were greater than the control in Year 2. The results indicate that cool-season grasses and legumes were the two groups that demonstrated less than significant advantages when compared to the control with only mycorrhizal inoculated plots.

Differences in Warm-Season vs. Cool-Season Grasses

The treatments had a somewhat variable effect on warm-season grasses compared to cool-season grasses. In the first year the average number of warm-season grass seedlings was approximately 30% greater than the control in both the mycorrhizal and micronutrient plots and the combination treatment showing an increase that was just below the

threshold of statistical significance at $p=0.083$. In Year 2, percentage of basal coverage was used as the method of comparison for reasons discussed in the materials and methods section. Interestingly, only the mycorrhizal inoculated treatment demonstrated a significant increase in basal coverage (40% greater at $p=0.008$) when compared with the control. Both the micronutrient and combination treatments showed a 30% increase over the control in Year 2, but at $p=0.260$ and $p=0.155$ it was not statistically significant.

As the literature suggests, this may be a result of warm-season grasses receiving a greater benefit by being a more closely related symbiont than other plant guilds (Wilson and Hartnett 1998). If this is the case you would expect the gap to widen between both the control and the micronutrient treatments in subsequent growing seasons. It is also possible that there is no significant difference between micronutrient and mycorrhizae treatments in warm-season grass basal coverage and the slight differences seen in Year 2 are a result of randomness and small sample size.

Unlike the warm-season grasses, there were no significant differences in cool-season grass seedling numbers or basal coverage of either the mycorrhizal or micronutrient treatments when compared to the control. This finding concurs with the literature regarding mycorrhizae's effect on cool-season grasses which suggest that while

they also receive a benefit from the symbiotic colonization of their roots it is not as great as warm-season grasses although the mechanisms for this are not well known (Hartnett and Wilson 1999).

Differences in Legumes vs. Non-Legume Forbs

Year 1 data only showed a significant increase in the average number of total forb seedlings for the mycorrhizal treatment.

Furthermore, neither legume or non-legume forb seedlings showed any differences when compared to the control. However, in Year 2 there was an increase of approximately 80% in basal coverage of non-legume forbs in both treatments that included mycorrhizae. While not significant, the basal coverage percentage of legumes showed a strong trend ($p=0.063$) of an approximately 40% increase for both the mycorrhizal and micronutrient treatments but not in the combined treatment.

Both years of data indicate that mycorrhizal colonization has a positive effect on non-legume forbs regardless of the addition of micronutrients. The addition of micronutrients only showed no difference in seedlings were seen in the first year and a non-significant trend ($p=0.125$) of increased basal coverage in non-legume forbs was seen during the second growing season. This suggests that

micronutrients play less of a role in plant growth of non-legume forbs when compared to the other plant guilds in this study.

Inoculation Success

The results for arbuscular mycorrhizal root colonization shows that the mycorrhizal inoculant significantly increased the percentage of roots colonized by fungal colonies. However, there was also mycorrhizal colonization of both the control and micronutrient only treatments which did not receive the mycorrhizal inoculum. The most likely explanation for the fungal colonization on non-inoculated plots is that there were preexisting mycorrhizal spores in the soil. Another possibility is the non-inoculated plots were colonized by invasion across the 3 m buffer from adjacent inoculated plots. This explanation is less likely since mycorrhizal colonization was tested during the second growing season while the native prairie still had a relatively low root biomass limiting the opportunities fungal colonies to migrate across the buffer.

Effects on Non-Natives

Competition from non-native species can have a detrimental effect on the establishment of native prairie species that are planted during a reconstruction (Dyer 1999). We had hoped to see a correlation between the treatments and the relative competitive pressure of the non-natives. This was evaluated by weed seedling count in Year 1, weed basal coverage in Year 2, and total weed biomass in both Year 1 and Year 2. However, the entire research site had relatively low pressure from non-natives in Year 1 and extremely low pressure in Year 2 which made it difficult to determine if any of the treatments assisted the natives in competitive exclusion.

Conclusions

- All three treatments improved establishment of native prairie species over the course of the experiment.
- Combining mycorrhizae and micronutrients did not amplify treatment effects on the establishment of native vegetation.
 - It may even reduce the beneficial effects.
- Weed presence was not significantly lowered but this result may be due to low abundance of weeds throughout the study site.
- Maximum effect was typically seen with adding only mycorrhizae
 - However, it was rarely significantly different than the micronutrient treatment.
- Mycorrhizal colonization has a greater impact on the establishment and growth of warm-season grasses than cool-season grasses.

CHAPTER 5

STUDY APPLICATIONS

Management Implications

This study demonstrated that the addition of mycorrhizal inoculant slightly outperformed the addition of the micronutrient seed coating. However, at the time of this experiment, mycorrhizal inoculant (MycoApply Granular, Mycorrhizal Applications Inc.) cost approximately \$200 per acre to add to the seed mixture. In contrast, the micronutrient seed coating used (NutriPlant SD 0-0-0, Amway Global) cost just \$0.29 per acre, making it a financially prudent option to include in seed mixtures to improve initial native plant establishment.

It is important to remember that this was only a two year study in prairie establishment. The addition of mycorrhizal inoculant would presumably continue to benefit the native plants as the prairie progresses through its early successional stages. Since the micronutrient powder was applied directly to the seeds this treatment will not supply a continued advantage to the native plants after the first few growing seasons.

The results of this experiment show that there is no positive cumulative effect of adding both micronutrient seed coating and mycorrhizal inoculant. In fact, in some instances the data suggest the

combination of the two treatments may have limited the effectiveness of the mycorrhizae. During the first year of establishment, the average number of total native seedling, total grass seedlings, warm-season grass seedlings, and the average number of forb seedlings of combined treatments were not significantly greater than the control while the treatment of just mycorrhizal inoculant was significantly higher. This continued in Year 2 for the basal coverage of warm-season grasses. Given the increased cost, with no significant benefits to prairie seeding success, using the combination treatment during reconstructions is not recommended.

The literature suggests that arbuscular mycorrhizal colonization disproportionately benefits warm-season grasses over cool-season grasses (Wilson et al. 2001). The literature also suggests that when certain nutrients are in abundance the plant-fungi relationship can be commensalistic or even parasitic in nature due to the associated costs to the plant of supplying the mycorrhizae with carbon and the nutrients not being a limiting factor to plant growth (Paszkowski 2006).

In this study, the inoculation, and subsequent increase of mycorrhizal colonization, provided a net benefit to the early establishment of planted native species in this tallgrass prairie reconstruction. This is a logical result since arbuscular mycorrhizae are an integral component of natural soil biota and are typically in reduced

populations in highly altered lands such as row crop agricultural sites. This study has demonstrated that colonization of arbuscular mycorrhizae aid in the early growth of native tallgrass prairie species. It has been determined that the chemical signatures of mycorrhizal hyphae elicits root branching so plants can presumably seek out colonization (Buee et al. 2000). Therefore, it is conceivable that these same chemical signatures may aid in seed germination in tallgrass prairie species but further studies are needed to investigate this idea.

Improvements to Experimental Design

Baseline soil nutrient levels were not determined for this study and potentially could have been useful in explaining the results of this experiment. Mycorrhizal colonization rates were not tested during Year 1 to confirm that native seedlings had been colonized during the earliest stages of development. There were many instances in this experiment when results were not considered significant at a 5% confidence interval but were marginally significant with p-values less than 0.10 but greater than 0.05. The best way to alleviate this would have been to increase the sampling size to increase statistical power.

Also, it would be preferable in future studies to use consistent methods of evaluating establishment from Year 1 to Year 2. Although

Year 1 seedling count data is valuable information to collect, I found it difficult or almost impossible to differentiate individual plants in subsequent years. This particularly applies to sod-forming grasses that may begin to reproduce rhizomonously or when two seedlings germinate and grow in close proximity to each other. There is also no way to account for Year 2 seedlings that are products of seed produced by the native plants in Year 1 which would over-represent annuals and biennials like *Chamaecrista fasciculata* and *Rudbeckia hirta*. For these reasons, it would be advantageous to use basal coverage over individual seedling or plant counts for all years of an establishment study.

Further Studies

Although the results from this study demonstrated benefits to early plant establishment in a tallgrass prairie reconstruction at this particular research site, it would be highly beneficial to reproduce these results across multiple research sites. Previous research has suggested that the nature of the fungal-plant relationship can vary depending on nutrient and resource availability, particularly in phosphorus, water, and micronutrients (Liu et al. 2000; Johnson et al. 1997). Even though this experiment utilized a split-block design it was conducted at one site with both blocks being adjacent to each other and in the same soil type.

This site had previously been in row crop agriculture, a continual disturbance which may have had an effect on the ambient level of naturally occurring mycorrhizae. Previous land usage also affects the amount micronutrients present in the upper soil profile where prairie seeds germinate and begin early growth (Farooq et al. 2012). Fungicides, herbicides, pesticides, and fertilizers have been shown to have a negative effect on fungal populations (Wilson et al. 2001) and future studies should determine baseline data for both mycorrhizal colonization of existing vegetation and ambient levels of micronutrients since results may vary based on the land usage history of a study site.

The mycorrhizal inoculant that was used in this study included the generalist mycorrhizal species *Glomus intraradices*, *G. mosseae*, *G. aggregatum*, and *G. etunicatum*. The species of mycorrhizae that colonized the plant roots in this study were not identified to genus and species and only percent colonization was determined. In future studies it may be beneficial to identify each to the species level. This would give an idea of the relative contribution of each fungal species in the inoculant. It would also determine if the mycorrhizal species present in the control and micronutrient plots were the same or different fungal species than those found in the inoculant. This study site had been in agricultural production and sprayed with glyphosate during the growing season prior to this experiment and there was little to no vegetation

present at the time of seeding. As a result, it was not possible to determine colonization percentages on existing vegetation to establish a baseline for the presence of mycorrhizae. It would be recommended to establish this baseline in future experiments if possible and perform annual colonization percentage testing to monitor the rate of spread. If planning an experiment using mycorrhizal inoculant that persists longer than two years it would be recommended to widen the buffer strips between inoculated and non-inoculated plots as well as routinely applying fungicide in the buffer strips to prevent possible fungal migrations.

Another study that should be performed is a study of the effect on germination for all three treatments. At this point it is unclear if the presence of micronutrients or mycorrhizae increases the germination rate of the native species that were included in the seed mix. The effect of individual micronutrients on germination has been studied (Farooq et al. 2012) but it is unclear if combination of the micronutrients used in this experiment has any combined effect. The presence of mycorrhizal hyphae can induce root growth and branching through unknown chemical signaling. It is possible that mycorrhizal spores could have a similar effect on seed germination when present.

The production of viable seed is commonly used to measure fecundity which is the true measure of the overall success of an

individual plant. This experiment demonstrated a significant increase in the number of flowering plants of the legume *Chamaecrista fasciculata* during the first growing season. Although a limited sample, it could support the idea that colonization of mycorrhizae may have effects on the production of viable seed. Future studies should examine seed production of select species of warm-season, cool-season, legumes, and non-legume forbs to determine if they are benefitted by mycorrhizal colonization. Despite the controllability of greenhouse experiments, this should be performed as a field experiment that utilizes a mixture of species to replicate the natural processes of a native prairie.

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APPENDIX A
SPECIES CODE FOR RAW DATA

Planted Native Species		CODE
Grasses		
Big Bluestem	Andropogon gerardii	angi
Side-oats Grama	Bouteloua curtipendula	bocu
Switchgrass	Panicum virgatum	pavi
Little Bluestem	Schizachyrium scoparius	scsc
Indian Grass	Sorghastrum nutans	sonu
Tall Dropseed	Sporobolus asper	spas
Prairie Brome	Bromus kalmii	brka
Canada Wildrye	Elymus canadensis	elca
Forbs		
Leadplant	Amorpha canescens	amca
Milk Vetch	Astragalus canadensis	asca
White Wild Indigo	Baptisia leucantha	bale
Partridge Pea	Cassia fasciculata	cafa
Purple Prairie Clover	Dalea purpurea	dapu
Showy Tick Trefoil	Desmodium canadense	deca
Round-Headed Bush Clover	Lespedeza capitata	leca
Thimbleweed	Anemone cylindrica	ancy
Prairie Sage	Artemisia ludoviciana	arlu
Butterfly Milkweed	Asclepias tuberosa	astu
New England Aster	Aster novae-angliae	asno
Prairie Coreopsis	Coreopsis palmata	copa
Pale Purple Coneflower	Echinacea pallida	ecpa
Rattlesnake Master	Eryngium yuccifolium	eryu
Ox-eye Sunflower	Heliopsis helianthoides	hehe
False Boneset	Kuhnia eupatoriodes	kueu
Rough Blazingstar	Liatris aspera	lias
Wild Bergamot	Monarda fistulosa	mofi
Wild Quinine	Parthenium integrifolium	pain
Foxglove Beardtongue	Penstemon digitalis	pedi
Prairie Phlox	Phlox pilosa	phpi
Common Mt. Mint	Pycnanthemum virginianum	pyvi
Yellow Coneflower	Ratibida pinnata	rapi
Black-eyed Susan	Rudbeckia hirta	ruhi
Compass Plant	Silphium laciniatum	sila
Stiff Goldenrod	Solidago rigida	sori
Prairie Spiderwort	Tradescantia bracteata	trbr
Golden Alexanders	Zizia aurea	ziau

Non-Planted Species		CODE
Siberian Elm	<i>Ulmus pumila</i>	ulpu
Green Foxtail	<i>Setaria viridis</i>	sevi
Dandelion	<i>Taraxacum officinale</i>	taof
Amaranth	<i>Amaranthus sp.</i>	amsp
Silver Maple	<i>Acer saccharinum</i>	acsa
Buttonweed	<i>Abutilon theophrasti</i>	abth
Mallow	<i>Malva neglecta</i>	mane
KY Bluegrass	<i>Poa pretensis</i>	popr
Yellow Nut Sedge	<i>Cyperus esculentus</i>	cyes
Mulberry	<i>Morus rubra</i>	moru
CA Goldenrod	<i>Solidago canadensis</i>	soca
Lamb's Quarters	<i>Chenopodium album</i>	chal
Yellow Sweet Clover	<i>Melilotus officinalis</i>	meof
Quackgrass	<i>Elymus repens</i>	elre
Common Cinquefoil	<i>Potentilla simplex</i>	posi
Soybean	<i>Glycine max</i>	glma
Wood Sorrel	<i>Oxalis stricta</i>	oxst
Cheatgrass	<i>Bromus tectorum</i>	brte
Purslane	<i>Portulaca oleracea</i>	pool
Marestail	<i>Conyza canadensis</i>	coca
Wild Plantain	<i>Plantago major</i>	plma
Field Bindweed	<i>Convolvulus arvensis</i>	coar
Red Clover	<i>Trifolium pratense</i>	trpr
Common Ragweed	<i>Ambrosia artemisiifolia</i>	amar
Carex Sedge	<i>Carex sp.</i>	casp
Sow Thistle	<i>Sonchus oleraceus</i>	sool
Black Nightshade	<i>Solanum americanum</i>	soam
Knotweed	<i>Polygonum aviculare</i>	poav
crabgrass	<i>Digitaria ischaemum</i>	diis
Mustard species	<i>Barbarea sp.</i>	basp
Virginia Creeper	<i>Parthenocissus quinquefolia</i>	paqu
Shepard's Purse	<i>Capsella bursa-pastoris</i>	cabu
Cottonwood	<i>Populus deltoides</i>	pode
Peppergrass	<i>Lepidium virginicum</i>	levi
Smooth Brome	<i>Bromus inermis</i>	brin
Canada Thistle	<i>cirsium arvense</i>	ciar
Aster specie	<i>Aster sp</i>	assp
Prairie Ragwort	<i>Senecio plattensis</i>	sepl

Non-Planted Species Cont.		CODE
Heath Aster	<i>Symphotrichum ericoides</i>	syer
Common Violet	<i>Viola sororia</i>	viso
Giant Ragweed	<i>Ambrosia trifida</i>	amtr
Witchgrass	<i>Panicum capillare</i>	paca

APPENDIX B
SEEDLING COUNT RAW DATA

Plot #: 5A-T1	Date: 7/29/09														
Sample #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Grasses															
ange	2	1	1	2		1	3		4	2	2	3			6
bocu	2	1	3	4	1	4	5		3	1		2	1	1	3
pavi			1	1			1						1		
scsc	1		1	1		1									1
sonu			1	1		1	2				1		1		
spas		1													
brka										2					1
elca						1	1		1		1	2		2	1
Forbs															
amca													1		
asca	1			2		1	1								1
cafa			1												
dapu		1	1	1	1	1	5		1					1	2
deca	1						3							2	1
leca	1														
astu						1									
ecpa															1
hehe														1	
mofi				1											
rapi						1							1		
ruhi				1					1		1	1			
ziau						1									
Other															
amsp	1	4	1	1	1		2	1				7		8	1
popr	7														
acsa		4													
ulpu				1	1	1	1								
brte					1										
pool							1								
taof								3							1
chal								1	2	3					3
coca										1					
glma										1					
plma											1				
coar													2		

Plot #: 6A-T1	Date: 7/29/09														
Sample #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Grasses															
ange	3	1	1		1	3			3		2	1	3	1	2
bocu	1	2	2			3			1		1	3		1	1
pavi	1			1											1
scsc		1	1		1						1				
sonu	2	1		3		2			1	1		1	2		
spas															1
elca			2	2		1		1	2		1	3	1		1
Forbs															
asca													1	1	
dapu		1		1					2			2	2		5
deca								1						1	1
arlu								1							
astu									1			1			
hehe				2	1										
mofi								1							
rapi	1								1				1		
ruhe	1			1							1	1	1		1
Other															
acsa	1			1							2		1		
amsp	1				1		1								
soca				1											
abth				1											
ulpu				2											
moru										1					
mane													1		

Plot #: 11A-T1	Date: 7/30/09														
Sample #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Grasses															
ange	1	1	2	3		1	2			2		1	3	1	1
bocu	3	3		1	1		2	1		1	1		2	3	4
pavi											1				1
scsc	2							2							
sonu		1	1		1	2								2	
elca						2	1				1	1	1		
Forbs															
asca		1				2									
cafa				1	1	1					1				
dapu	1	1	4		3	1	1	1			1				3
deca								1				1	2		
leca														1	
astu							1					1			
ecpa							1								
hehe	1						1	1		1				1	2
kueu							1				1				
mofi					1										
rapi	2		1			2	2					2		1	1
ruhi	1		2		3	2									
trbr														1	
ziau											1				
Other															
amsp			1	1	1		1	3	3	2	2				
oxst							1	1							
acsa									1					2	
coca							1	2							
ulpa	1		1		1					2					
sevi	1														
pool	1														
sool												1			

Plot #: 1B-T1	Date: 7/28/09														
Sample #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Grasses															
ange	2	1	2	1	2		3	3	3				4	1	2
bocu		2		3		4	3	2	3	3	1	4	2	5	2
spas									1						
pavi		1													
scsc							1					1			1
sonu					1		1								
elca			1				1		1		2			1	1
brka						2									
Forbs															
asca							1		1						
dapu							4	1		2		1	1		
deca					1									1	
astu									1		1				1
hehe									2						
rapi									1					1	
ruhe									1		1	1			
trbr													1		
Other															
mane	1														
amsp	3	5	2	1	5	3	5	3	6	1	11	2	3	1	
taof	2								3			1			
acsa			2												
ulpu				1		1	1			1		1		1	
sevi											1				

Plot #: 7B-T1	Date: 7/28/09														
Sample #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Grasses															
ange	1	1	3	2	4	1		4	2	2		1	1		
bocu	2		2	3	2		1		1	1	4		1		4
scsc		1		1				1	1						
sonu	2		1	1	1			1					1		
brka			1	1											
elca			2	1			1	1	2		1		1	2	1
Forbs															
asca			1					1	1				1		
cafa			1		1										
deca		1	1	1			1				1		1		1
ecpa	1														
astu		1	2						1			1		1	1
dapu				1	2			1				1			
hehe										1					
kueu	1		1						1						1
rapi	1				1			1	1						
ruhi	1			1	2				2		1		2		
sila								1							
Other															
moru	2	1					1								1
mane	3	7	5	1	5	5			4	10	7	7			
ulpa	2		1				4				2	1	1	1	1
acsa		1					1						1		
amsp			1		1	1	1	2				3	1		
pool											1				

Plot #: 4A-T2	Date: 7/29/09														
Sample #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Grasses															
ange	3	5		3		1	4	3	1	2	3	4		4	
bocu	3	2		6		3		1		4	4	4		6	2
pavi			2								1				
scsc	1	1	1	1		1			1	1	1	1			
sonu	3		1	1			1	3	1	2	2			1	
elca	1	2		1			2	1		1		2			
Forbs															
asco		1										1			
cafa									1	1					
dapu	2	1					1	1	2	1	2				
deca		1		5			1				1	1			
astu	1	1		1							1				
hehe	2		1	1		1				2				1	
rapi	1			2				1				3		1	
ruhi		2		3			1			1	2			1	
sila														1	
trbr	1														
Other															
elpu	1	1		1				1							
amsp	5	5	1	1	1	5	2		4	1	2	3	4	5	1
taof	1							1				1	1		1
chal					2										
glma					1										
sevi							1						1		
acsa								1				1			
moru														1	
oxst											1				

Plot #: 8A-T2	Date: 7/30/09														
Sample #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Grasses															
ange		4		1		1	2	1	1	2	5	5	1	1	
bocu	5	2	3	6	3	5	3	4	4		8	2	1	3	5
pavi															1
scsc		1			2	1			2					1	
sonu	2	2	1			1			1			1		2	1
elca	1		1	2	2	3		2	1	2	2	1		2	2
Forbs															
asca		1			1	1						1			1
cafa				1								1			1
dapu	3	3	1			1	2	3		1				1	5
deca		1		2		3	1		1	1			1	1	1
leca		1			1			1	1						1
astu	1			1		2				1					
eryu									1						
hehe					1	2	1	2			1	2			
kueu				3	1										
mofi					1										
rapi		1	1	1	1	4		3	1						1
ruhi	1	1			1	2						1		1	1
Other															
amsp		1	1		8	1	1			1	1	1		2	1
acsa			2		1	1									
oxst					1										
amar							1								
sevi							1								
taof												1			1
ulpu										1	1				

Plot #: 5B-T2	Date: 7/28/09														
Sample #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Grasses															
ange	3	2	1	1	2	4	2	3	1		1	2		3	2
bocu	3	2	1		3	2	4		2	4		6		3	
pavi			1			2									
scsc	3			1					1			2			
sonu		2	1	1			2		1						1
brka		1											1	1	
elca	1	1								1		2			1
Forbs															
amca						1							1		
asca	2				2	2				1	1				
dapu	1					3	2		1			1			2
deca	1	1	1			3			1	1		1		1	1
astu						3	1								1
ecpa															1
hehe		2	1			2	1		1	1					1
kueu			1				2		1						1
rapi			1		1			1							
ruhi					2					2		1	1	1	
Other															
amsp	3	1		1	2		3	3		1					
ulpa	1				2		3	2	1	1	3		2	1	4
amar		1													
mane		1		1			1								
taof		1					1	1			1				3
acsa			1	1					1						1
diis					1										
plma													1		

Plot #: 10B-T2	Date: 7/28/09														
Sample #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Grasses															
ange	3	1	1	1	3	1	2	1	2	2	5	6	5	3	2
bocu	2	1	1	5	4		2	3	2	1	7	4	2	1	
pavi							2			1					1
scsc			1			1							1		
sonu	1	2	1	6	1		2	2	1	1	2	1	1		3
spas						1									1
brka										1		1			
elca						1			2	1	2		2		2
Forbs															
asca					1	1	1					2	1	1	2
cafa					1	1					1	1	1		1
dapu	3	1		4	2	1	7	1			2	4	2	2	2
deca	2		1	1		1	1					2	3		
astu							1				1				1
ecpa							1								1
hehe	1			3	1	1	1			1			2		1
kueu	1			1		1									
rapi						1	1	1			1	3	1		2
ruhi	1				2	2	3			1		3	1	1	1
Other															
amsp	1	1			1	1			1		1			1	
taof	1									1					
mane		1	1							1					1
acsc		1													1
pool				1											
cabu						1									
ulpu												1			1

Plot #: 10A-T3	Date: 7/30/09														
Sample #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Grasses															
angi	3	2	1	4	3	1	1		1	1	2	2	2	1	
bocu		1	1	7	2		3		5	4	1	2	1	6	1
pavi							1					1			
scsc			1	1			1				1				1
sonu				2	2	4			1	1		1	1	2	
elca	5			2		1	3	1	1		2	1			
Forbs															
asca				1				1		1		2	1		
cafa													1		2
dapu	2	1	2	1		5				1	1	2	2	1	
deca				2	1	1	1	1	2				2		
astu			2			3		1				1	1		1
hehe						1			1					1	
kueu			2												
rapi						2			1				1		1
ruhi		1	1			1	1		1						1
silu											1				
Other															
popr	1												1		
amsp		3			2			2					1		1
acsa		1			1							1	1		
ulpu						1	2			2	1				

Plot #: 12A-T3	Date: 7/30/09														
Sample #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Grasses															
ange	1		1	2	1	3		6	2		1	2	2	1	2
bocu	3	1	3	4	3	2	2	1	5	3	2	1		6	3
pavi				1						2					1
scsc		1					2			1		1			
sonu		1	2	1	2	1	1	1		1	1		1	1	1
brka							1			1					
elca	2	1									2	1	1	1	
Forbs															
dapu		1			1			2	2		1	1		2	4
deca	1			1	3			1			1	1	1		
leca											2				
astu		1			1		1								
ecpa		1					1			1					
hehe	2	1							1		1	1	1		
kueu											1				
mofi												1			
rapi	1	1		3	1				1	1		1			2
ruhi	2			2						1		2			4
Other															
ulpa		1			1					1		1	1	1	
mane		1													1
taof	1	1						1					1		1
pool			1								1			1	
amsp				1	1	1	2		1	1		1	1		1
sevi	1														
moru					1						1				
acss				1							1				

Plot #: 2B-T3	Date: 7/27/09														
Sample #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Grasses															
ange	3	4		2	3	1	1	3	1	2	2	2		1	2
bocu	4		2	2		2	3	1	1		3		1	1	
pavi	1	1			1						1		1		
scsc			1				1				1		1		
sonu		1	1									1			1
brka			1												
elca	1	2	1	3	2	1					2		1	1	3
Forbs															
asca	1				2										1
dapu	3	1			2			3	1		1		1		1
deca		1	1								1				
cafa						1									
astu	1														
hehe			1	2		1									
mofi	1				1										
rapi		1	1		2										1
ruhi	1	1			1	1									
Other															
soca	3		1												
acsa	1			2		1				1			2		
ulpu	3		3	2	4	4	3	6	1		5	1	2	1	5
amsp		1	1	3		1		1			1		1	1	1
taof		2	3	6	2	1	2		6				2	2	1
mane			1									1			
sevi									1	3					

Plot #: 2A-T4	Date: 7/29/09														
Sample #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Grasses															
ange	5		5	2	2	5	1	1		2	2		3		1
bocu	3		2	1		3		4		2	1	2	1	2	1
pavi									1			1	1	1	
sonu				2	1	1				1	1	1			
spas					1						1				
brka															1
elca	2		1	1	1					2	1	1	2	1	2
Forbs															
asca	2														
dapu	1		1	2										1	
deca	2					1					2		1		2
leca					2										
astu	2				1									1	1
cafa	1					2									1
ecpa				2		1				1		2		1	
hehe				1						1	1				
kueu						3							1	1	
rapi	1					2						1	1		
ruhi												1		1	1
sori							1						1		
trbr														2	
Other															
ulpu	4		1			2					1	1			2
acsa	2		2	1	1	2	1				1		1	1	2
taof	3				1			1	1		4		1		
amsp		2			2				1	1	4	2	7	1	
meof					1										
chal		2													
sevi		1		1											
soca		1													
elre				1											
moru									1						

Plot #: 3A-T4	Date: 7/29/09														
Sample #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Grasses															
ange	1	1		2	3	2			3	2	3	3	1	1	1
bocu	5	2	4		3	2	1	3	5	2	3	6	2		
pavi	1										1	1		1	
scsc			1		2										1
sonu	1			2		1		1	1		1	2	1	1	
brka									1						
elca	1			2	3	2	4	1	1			1	1	1	
Forbs															
amca							1								
asca		1					1				2		3		
cafa			2									1			
dapu	1	1					1			1	1	2	2	1	1
deca	1	2						1			2	1			1
astu														1	
hehe								1					1		
mofi							2								
rapi	1						1	1							
ruhi	1						1		2		1		1		
Other															
acsa	3	2		1	1			1			1	1		2	2
amsp		2		3	5	4			1		2	4	2	1	8
ulpu		1	1						1			1			
popr			1												
sevi									2						
posi									1						

Plot #: 7A-T4	Date: 7/29/09															
Sample #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
Grasses																
ange	6	5		2	1	4	3		3		2		4	1	2	
bocu	2			4	4	3		4	2		2	1	5	1	3	
pavi										2				1		
scsc			1		1	1		1			1				1	
sonu				1		1			2	1					1	
spas										1				1		
brka		1								1			1			
elca		1			2				1	1	1		2	2	2	
Forbs																
asca	1			1		1		1					1			
cafa		1		1										1		
dapu	3	1			1					2					1	
deca													2		1	
astu	1			1											1	
ecpa	1															
hehe		1				1										
kueu				1											1	
rapi			2	1			2								1	1
ruhi			1	2			1								1	1
sila											1		1			
Other																
abth	1															
amp		1	1	2		1				1	10	3				
moru											1					
acsa		2	1	2	1	2	1							2		
ulpu		2				1										
trpr				1												
mane												1				

Plot #: 11B-T4	Date: 7/29/09														
Sample #	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Grasses															
ange	4			1	4		2	2	5	1	4	3	3		3
bocu	2				2	3		1	3	2	1	1	4		8
pavi															1
scsc															2
sonu					2		1								
brka													1		
elca	1						2	3							1
Forbs															
asca	1				1			1							1
cafa					1								2		
dapu	4				3		3		1		7			1	2
deca					2		1	2			1				2
astu								2							
ecpa															1
hehe					1		1	1							2
kueu					1						2		1		1
mofi								2							
rapi					1			2			2				1
ruhi	2					1			1		3				1
Other															
taof	1		1		2					2		1		2	1
amsp	4				3	3	1	1	1	1	1	2	2	2	1
ulpu		2	3	3		1	1	3	2		2	1		2	
acsa		1													
pool													1		

APPENDIX C
FIRST YEAR BIOMASS RAW DATA

Block	Treatment	Grass Biomass (g)					Forb Biomass (g)					Weed (g)				
1	1	2.1	1.2	0.2	5.7	4.9	0.1	0.3	0.2	0.6	0.1	3	24.8	0.3	21.7	4.3
1	1	2.2	2.1	5.4	1.2	4.9	1	1.4	0.3			0.1				
1	1	5.8	4.9	0.6	2.1	13.1	2.9	1.1	0.3	0.7	0.5	4.5	23.4	6.3	27.2	
2	1	3.5	1.4	1.3	2.8	2.1	0.2	0.1	0.2	0.3		16.1	32	14.8	6.6	
2	1	5.6	0.9	1.2	1.9		1.4	1.2	4.7	3.6		0.4	0.5	10.2	1.8	1
2	1	1.2	0.8	3	6.2		0.5	0.1	1.5	0.9		6	16.4	1.8	5.3	1.6
1	2	4.3	5.8	9	3.5	0.5	1	0.5	3.6	0.8	0.6	10.2	1.5	0.7	16.5	5.5
1	2	2.3	5.5	4.7	3.2	10.3	3.7	5.8	0.2	0.6	0.6	5.7	1.7	3.5	8.2	0.9
1	2	6.7	6.7	3.8	12.6	15	2.5	3.3	1.3	0.8	3.5	1.9	0.9	4.6	0.3	4.8
2	2	1.4	0.5	0.4	0.1	0.1	0.1	0.2	0.1	0.1		1	31.9	2.1	1.5	14
2	2	3.2	0.5	4.1	2.5	5.9	1.5	0.1	1.2	1.5		8.1	3.1	5.5	12.1	20.5
2	2	4.2	19.4	6	4.3	5.9	0.9	1.9	1	4.5	0.5	2.4	4	0.1	0.1	2.8
1	3	1.8	2.6	2.1	0.3	1.2	0.2	0.8	0.1	0.8	0.2	3.1	2.9	2.2	1.8	6.7
1	3	3.6	13.1	0.4	10	0.8	0.5	2.1	1	3	7	0.4	0.8	1.2		
1	3	3.8	4.9	5.6	3.1	3.9	0.6	6	2.2	0.4		1.8	0.2	5.1	9.2	0.6
2	3	1.8	0.5	0.2	1.3	1.2	0.6	0.9	0.2			19.9	24.9	6.6	3.2	17.9
2	3	3.5	3.2	2	2.1	2	0.2	0.6	9	0.1		0.6	23.4	22.4	12.5	
2	3	5.5	5.3	5.5	8.4	4.2	1.3	0.2	0.7	0.1	1.5	8.4	11.2	3.7	5.4	0.5
1	4	5.3	0.7	0.2	2.8	1.5	1.6	0.2	1.9	1.1		1.5	0.1	5.3	2.2	7.9
1	4	2.7	2.1	2.1	2.8	1.8	1.8	3.7	1			1.6	1.8	14.7	0.6	26.4
1	4	4.5	7	1.2	0.9	5	0.1	0.5	1.1	0.3		0.4	2			
2	4	3	6.5	2.2	1	2.4	2.6	1	3.8	0.2		0.1	12.3	7.7	1.3	5.2
2	4	8.5	1.6	0.9	4.2		1.5	1.1	1.4	0.1	2.6	12.1	3.6	1.6	12.2	7.5
2	4	0.1	8	1.2	3.3		3.5	0.3	2.1	0.1		5.2	2.4	2	6.2	0.5

APPENDIX D
FLOWERING TIME RAW DATA

BLOCK	TRMNT	Part Pea	BE Susan	TOTAL NATIVE
1	1	39	24	64
1	1	36	6	42
1	1	31	3	35
2	1	37	18	55
2	1	44	4	48
2	1	40	2	42
1	2	72	51	123
1	2	68	27	95
1	2	49	3	52
2	2	73	23	97
2	2	62	18	81
2	2	47	3	51
1	3	52	34	88
1	3	64	18	82
1	3	54	7	61
2	3	66	42	109
2	3	56	19	77
2	3	22	2	24
1	4	64	39	103
1	4	57	25	82
1	4	71	6	77
2	4	57	28	85
2	4	51	2	53
2	4	48	3	51

APPENDIX E
YEAR TWO BASAL COVERAGE RAW DATA

Plot #: 11A-T1	Date: 7/1/10									
Sample #	1	2	3	4	5	6	7	8	9	10
Grasses										
ange		0.200			0.100			0.100	0.100	0.200
bocu	0.200			0.200	0.200		0.056			
pavi					0.130					
scsc							0.075			0.081
sonu	0.100	0.100	0.130	0.100					0.130	
brka	0.063		0.031					0.100		
elca	0.130	0.088	0.250	0.100					0.200	0.130
Forbs										
asca			0.019							
cafa		0.025	0.025	0.006			0.056		0.006	
dapu										0.006
deca		0.019	0.025						0.025	
astu					0.006					
ecpa		0.019								
hehe		0.019						0.130		
kueu	0.056	0.031								0.050
rapi			0.019						0.063	
ruhi						0.063				
sori								0.019		
Other										
ulpu		0.019				0.013		0.063		0.025
acsa			0.025		0.025	0.006	0.025			
taof		0.200			0.200		0.130			
ciav			0.031							
popr										0.088
sepl	0.130									
assp								0.044		
pode	0.013						0.013			
coca							0.025			
glma							0.025			

Plot #: 7B-T1	Date: 6/30/10									
Sample #	1	2	3	4	5	6	7	8	9	10
Grasses										
ange			0.100		0.100			0.100		
bocu			0.200			0.100	0.200			0.250
pavi							0.130			
scsc										
spas										
brka									0.031	
elca	0.200	0.310	0.130					0.100	0.310	0.100
Forbs										
asca	0.075	0.200	0.056					0.050		
cafa	0.006		0.025	0.019				0.013		
dapu							0.006			
astu							0.019			
hehe	0.019			0.130	0.069	0.050			0.063	
phpi	0.006									
rapi			0.025			0.038				
ruhi	0.006					0.130				
сила	0.006									
Other										
ulpu		0.044			0.025	0.056	0.031	0.088	0.044	
acsa	0.019	0.006								
ciar										0.100
taof			0.019							0.310
abth	0.006									
soca						0.044				
sool				0.019						

Plot #: 4A-T2	Date: 7/1/10									
Sample #	1	2	3	4	5	6	7	8	9	10
Grasses										
ange	0.200	0.200	0.200			0.200	0.200		0.130	0.250
bocu	0.200	0.100	0.200	0.250			0.130	0.200	0.130	0.130
pavi								0.200		
scsc					0.200	0.100	0.130	0.094		0.100
sonu	0.310	0.310		0.200	0.200		0.400			0.200
elca			0.400	0.400	0.250	0.200			0.250	0.100
Forbs										
amca			0.006							
asca	0.094			0.063	0.044		0.130		0.100	
cafa	0.013		0.006	0.006	0.019	0.006	0.013		0.044	0.019
dapu	0.006		0.013		0.006					
deca		0.038	0.056		0.050				0.088	
leca										0.013
astu					0.013					
ecpa								0.025		
hehe	0.100	0.075		0.044					0.075	
kueu	0.063	0.056				0.069				
mofi								0.050		
pain									0.006	
rapi	0.031							0.100	0.050	0.063
ruhi	0.100	0.250	0.038	0.100	0.200			0.250		0.081
silu						0.013		0.038	0.006	
Other										
ulpu		0.038						0.019	0.019	
acsa						0.006	0.025	0.006		
taof								0.019		
popr			0.025			0.013	0.013			

Plot #: 8A-T2	Date: 7/1/10									
Sample #	1	2	3	4	5	6	7	8	9	10
Grasses										
ange	0.100	0.100	0.200	0.200	0.310					0.100
bocu	0.200			0.310	0.200	0.081		0.100		0.200
pavi					0.200					
sonu	0.200		0.310					0.100		
spas										0.200
brka				0.031						
elca		0.800	0.100	0.450	0.200	0.100	0.700		1.830	
Forbs										
asca			0.056			0.044		0.063		0.056
cafa		0.025	0.044			0.075	0.025	0.044		0.050
dapu			0.006				0.019	0.013		
deca				0.044						
leca						0.006				
astu								0.006		
hehe	0.170	0.088	0.006	0.063		0.081		0.031		
kueu		0.081								
mofi						0.044				
pain							0.013			
rapi			0.075		0.019	0.050		0.038		0.081
ruhi	0.200		0.100			0.130		0.250		0.130
sori			0.019							
Other										
ulpu			0.019	0.019				0.013		0.019
acsa	0.006						0.019			
amar								0.006		

Plot #: 9A-T2	Date: 7/1/10									
Sample #	1	2	3	4	5	6	7	8	9	10
Grasses										
ange			0.075	0.400	0.200	0.130		0.310	0.100	0.200
bocu	0.130	0.056	0.200		0.200	0.250		0.130	0.200	0.200
pavi					0.250	0.200	0.100		0.200	
sonu	0.250	0.200		0.200				0.130		
spas						0.100				
brka									0.100	0.081
elca	0.400	0.130	0.400	0.200	0.130		0.130	0.200	0.250	0.310
Forbs										
amca							0.006			
asca					0.088	0.038	0.044		0.130	
cafa			0.013	0.013		0.013	0.013	0.013		0.038
dapu	0.013									0.006
deca							0.038			
leca			0.013							
astu		0.013								
asno				0.036						
ecpa							0.031			
hehe		0.081			0.031	0.094				0.088
kueu			0.019			0.025				
mofi	0.038			0.063						
phpi			0.006							
rapi	0.130	0.088	0.056	0.200	0.130	0.050	0.056	0.056	0.081	
ruhi	0.200			0.310		0.100	0.550			0.075
Other										
ulpu	0.019					0.019				
acsa		0.013	0.006	0.019			0.019			
soca								0.200		
popr							0.019			
ciar							0.025			

Plot #: 3B-T2	Date: 6/28/10									
Sample #	1	2	3	4	5	6	7	8	9	10
Grasses										
ange	0.038	0.200		0.310	0.320			0.044		0.250
bocu			0.630	0.400	0.600	0.063		0.450	0.130	0.056
pavi	0.056	0.630	0.056						0.088	0.200
scsc			0.800					0.075		
sonu	0.500			0.250		0.044		0.050	0.250	
brka	0.200								0.063	
elca	0.088	0.250	0.310	0.200		0.700	0.200	0.250	0.069	0.630
Forbs										
asca		0.160			0.056		0.120	0.056	0.075	
cafa			0.006	0.031			0.038	0.006		0.006
dapu			0.006	0.006	0.006	0.006	0.019		0.006	0.013
deca	0.013				0.019		0.031		0.025	0.031
astu			0.006							
hehe				0.025		0.013				
kueu									0.019	0.044
mofi							0.075		0.006	
phpi	0.006									
rapi		0.025					0.031	0.025		
ruhi		0.025		0.400	0.450	0.050	0.630	0.056		0.063
sori							0.019			
Other										
ulpu	0.044	0.081	0.038	0.056	0.063	0.050	0.094	0.044	0.075	0.150
taof	0.006		0.006	0.013		0.006	0.006			
acsa						0.013				
assp			0.013		0.006					
popr			0.013			0.006			0.031	
brte					0.006					
amar	0.006	0.013				0.013		0.019		
sepl						0.031				

Plot #: 2B-T3	Date: 6/30/10									
Sample #	1	2	3	4	5	6	7	8	9	10
Grasses										
ange		0.200		0.200			0.088		0.200	
bocu	0.200	0.130		0.100	0.200	0.075		0.200	0.130	0.130
sonu		0.250	0.100		0.200		0.130	0.310		
spas										
elca	0.310		0.050	0.250	0.250	0.200		0.250	0.081	0.310
Forbs										
asca		0.094			0.050		0.063		0.088	
cafa	0.013	0.013		0.019	0.006	0.013	0.013		0.006	0.006
dapu	0.006	0.013	0.006							
deca	0.088	0.056	0.006		0.025	0.013	0.056			
astu				0.031						
hehe	0.031	0.031			0.013					
kueu					0.013		0.044			
rapi			0.050		0.025				0.069	0.038
ruhi		0.200	0.130		0.250	0.250				
сила								0.006		
Other										
ulpu	0.050	0.019	0.006	0.006	0.031	0.050			0.050	0.081
acsa		0.006	0.019							0.019
popr			0.013							
taof			0.025	0.025		0.031	0.019			0.025
coca			0.013							0.025
assp				0.006						0.013
sool			0.019							

Plot #: 9B-T3	Date: 6/29/10									
Sample #	1	2	3	4	5	6	7	8	9	10
Grasses										
ange			0.080	0.200		0.200	0.170		0.088	0.069
bocu		0.160	0.044	0.056		0.250	0.100		0.100	0.063
pavi					0.063		0.130			
scsc			0.088				0.094			
sonu		0.094		0.130	0.130	0.130	0.200		0.050	
spas					0.069					
brka							0.100		0.100	0.063
elca	1.200	0.250	0.600	0.200		0.800		0.740	0.400	0.630
Forbs										
asca			0.094		0.160		0.063		0.031	0.038
cafa	0.013		0.019	0.031	0.019	0.006	0.025	0.063		
dapu	0.006				0.006					
deca			0.006		0.019			0.031	0.013	
leca				0.006						
astu		0.006	0.013		0.006				0.013	
hehe	0.050						0.025			
kueu									0.013	
phpi				0.006						
rapi	0.044	0.056	0.063		0.044	0.013			0.013	0.013
ruhi	0.250		0.031		0.230	0.088	0.069		0.088	
slia		0.006								
Other										
ulpu	0.038	0.025	0.031	0.031	0.044		0.013		0.019	0.050
coca										0.013
acsa	0.025	0.006								
popl						0.006				
popr										0.019
amar	0.019								0.056	

Plot #: 6B-T4	Date: 6/29/10									
Sample #	1	2	3	4	5	6	7	8	9	10
Grasses										
ange	0.100	0.200			0.200	0.063	0.063		0.100	0.200
bocu	0.075	0.088		0.250	0.130	0.050		0.200	0.130	
pavi	0.094							0.310		
scsc			0.250			0.200				0.130
sonu	0.200		0.310	0.031	0.081					0.069
broka				0.094						
elca	0.031	0.160	0.400		0.056	0.550	0.075	0.310	0.100	0.250
Forbs										
asca		0.025						0.019	0.050	
cafa	0.013			0.006	0.031			0.025	0.006	
dapu	0.006	0.006			0.006				0.006	0.013
deca	0.013		0.006	0.019		0.031	0.031			
leca		0.006								
astu	0.006								0.025	
ecpa	0.006	0.006								
hehe	0.094	0.038		0.056			0.019			0.050
kueu				0.038			0.006		0.019	
mofi						0.025				
phpi	0.006									
rapi		0.050	0.100	0.019					0.081	
ruhi	1.100	0.075	0.200	0.310	0.069		0.310	1.000		0.200
Other										
ulpu	0.038	0.031	0.031	0.044	0.081		0.100	0.013	0.031	0.056
taof										0.013
acsa	0.006			0.013	0.006			0.013	0.006	0.006
moru								0.006		
viso								0.006		
amtr				0.006						
amar				0.019	0.019					

Plot #: 8B-T4	Date: 6/30/10									
Sample #	1	2	3	4	5	6	7	8	9	10
Grasses										
ange	0.100	0.130					0.130			
bocu	0.100	0.250	0.100		0.100	0.130	0.200			0.100
pavi	0.200							0.310		
scsc				0.088		0.088	0.130			
sonu			0.075	0.050	0.200	0.100		0.130	0.200	0.130
brka	0.063	0.025							0.038	0.025
elca		0.081	0.100	0.160		0.130	0.200	0.250	0.250	0.130
Forbs										
asca	0.063		0.025		0.050	0.200	0.100			0.038
bale	0.006									
cafa	0.013		0.006	0.019		0.013	0.013	0.025		
dapu									0.013	
deca	0.038					0.025	0.013	0.006		
leca	0.019									
astu						0.006			0.013	
hehe		0.056	0.025	0.200				0.044		0.050
kueu		0.050								
mofi								0.038		
rapi	0.019	0.075	0.019							
ruhi	0.200	0.031	0.600	0.075		0.130		0.200		0.130
sori			0.036							
Other										
ulpu	0.031	0.025	0.031	0.025	0.025		0.031	0.056		0.019
coca					0.013					0.006
taof			0.019	0.019						0.019
soca								0.006		
amar				0.025						

Plot #: 11B-T4	Date: 6/29/10									
Sample #	1	2	3	4	5	6	7	8	9	10
Grasses										
ange	0.100	0.400	0.056	0.130		0.130	0.130	0.250	0.220	0.088
bocu	0.200		0.094	0.130			0.100			0.200
pavi					0.063					0.130
scsc				0.100						
sonu			0.630	0.080	0.100	0.200			0.075	
spas										
brka				0.056						
elca	0.130	0.250			0.310	0.250	0.088	0.800		
Forbs										
asca	0.025			0.150				0.075	0.081	
cafa	0.006	0.069		0.013	0.025		0.038			
dapu		0.006		0.013	0.006				0.013	
deca			0.019		0.044				0.063	0.044
leca						0.013				
astu							0.013	0.025		
ecpa							0.006			
hehe	0.025			0.006			0.044			0.019
kueu					0.050					
rapi		0.019			0.019		0.025			0.038
ruhi	0.200		0.630						0.550	
trbr			0.025							
Other										
ulpu	0.025	0.019	0.044		0.019			0.031	0.013	0.019
acsa					0.006					
bode				0.006						
taof										
assp							0.031			

APPENDIX F
YEAR TWO RAW BIOMASS DATA

BLK	TRTM	Grass Biomass (g)						
1	1	112.0	49.7	80.2	104.5	18.3	89.8	34.5
1	1	44.7	61.2	32.4	5.3	31.3	8.2	0.0
1	1	11.4	52.5	25.8	51.1	31.2	52.9	26.6
2	1	60.6	29.0	8.0	48.6	13.1	15.7	29.1
2	1	42.9	82.3	15.4	59.0	28.5	7.9	57.8
2	1	50.5	28.0	45.1	89.2	25.6	73.2	30.9
1	2	304.8	39.5	25.1	87.0	12.5	149.7	35.8
1	2	47.4	33.0	99.9	76.2	26.7	33.2	71.2
1	2	123.6	48.2	56.2	36.1	98.3	37.0	108.0
2	2	18.5	132.6	28.2	40.4	65.3	18.0	76.0
2	2	66.6	55.6	71.1	15.6	78.7	48.5	17.1
2	2	47.5	20.0	43.1	30.8	27.3	46.3	43.0
1	3	80.1	36.3	36.0	62.9	24.0	177.7	12.5
1	3	56.3	95.2	27.1	65.3	55.9	60.2	78.9
1	3	52.4	134.2	30.9	62.4	1.7	60.3	82.6
2	3	28.8	100.0	84.0	29.5	99.6	4.0	43.3
2	3	33.5	33.1	66.2	46.4	37.5	55.1	57.8
2	3	25.3	12.5	63.6	27.3	125.4	136.8	91.6
1	4	124.4	17.1	32.5	11.2	49.8	40.8	94.7
1	4	115.2	55.1	137.0	140.9	40.7	115.6	30.0
1	4	48.9	43.9	54.3	58.1	53.3	45.0	93.7
2	4	19.9	19.2	35.9	29.3	44.1	25.4	69.1
2	4	47.2	44.1	42.4	41.4	138.8	61.2	34.1
2	4	59.5	115.6	14.0	15.4	14.1	83.0	28.0

BLK	TRTM	Forb Biomass (g)						
1	1	138.7	4.8	1.9	0.8	15.7	2.3	4.8
1	1	5.4	6.0	3.5	3.6	0.3	32.5	11.5
1	1	22.3	0.0	0.1	4.0	6.8	3.1	33.8
2	1	37.4	1.6	0.0	10.8	9.0	16.8	31.5
2	1	4.3	0.0	9.8	0.5	1.1	0.1	4.4
2	1	11.6	9.4	2.6	0.0	5.7	20.7	0.0
1	2	9.4	22.9	25.2	0.4	51.3	17.1	80.8
1	2	23.1	42.4	35.7	90.6	62.9	2.3	14.2
1	2	3.2	8.6	47.9	35.3	35.3	58.6	26.3
2	2	87.5	12.8	34.5	12.5	17.3	59.3	11.8
2	2	55.6	29.8	32.1	51.6	13.0	42.3	48.7
2	2	1.3	39.6	0.4	1.6	27.3	76.5	10.6
1	3	6.2	21.6	71.2	4.4	2.2	14.9	48.2
1	3	1.0	24.8	31.5	36.5	39.1	15.9	22.3
1	3	63.3	23.3	30.0	0.9	95.6	24.5	17.9
2	3	23.9	0.2	0.9	49.9	2.3	3.4	40.2
2	3	16.8	76.2	56.4	40.7	14.0	19.5	58.9
2	3	0.9	89.8	70.9	2.0	11.7	9.7	45.0
1	4	40.2	51.3	49.2	50.4	16.1	55.0	17.2
1	4	6.0	23.5	0.0	6.1	4.6	70.4	5.4
1	4	55.4	36.2	9.0	61.6	2.9	9.7	34.1
2	4	83.0	17.3	65.6	52.3	32.7	21.8	0.3
2	4	47.8	0.4	54.9	2.5	26.2	5.0	83.0
2	4	15.2	0.0	45.8	40.5	11.7	24.6	98.2

BLK	TRTM	Weed Biomass (g)						
1	1	0.6	0.7	7.4	0.9	3.7	0.4	1.7
1	1	3.3	0.0	3.6	7.7	2.3	5.0	0.4
1	1	0.0	3.3	0.0	14.0	1.2	0.0	3.6
2	1	2.0	2.0	5.8	3.0	4.1	1.3	1.3
2	1	0.4	1.5	0.0	4.0	0.2	0.0	0.0
2	1	0.2	5.1	2.8	9.5	1.7	0.0	2.5
1	2	0.0	0.3	0.6	0.0	0.9	6.1	0.0
1	2	0.2	0.6	0.8	0.0	2.2	10.2	0.0
1	2	0.2	0.0	0.0	1.2	1.6	0.4	0.0
2	2	0.0	0.0	0.4	0.0	7.0	0.6	0.2
2	2	2.5	3.5	0.1	0.9	3.9	2.3	0.2
2	2	2.5	3.3	3.1	3.5	2.4	6.1	3.3
1	3	11.5	16.7	1.1	7.9	4.6	3.2	22.3
1	3	17.3	0.0	0.0	7.4	0.0	0.0	1.1
1	3	0.0	0.0	1.4	1.1	3.0	0.0	0.0
2	3	4.5	0.0	1.2	2.6	10.9	2.3	0.4
2	3	2.1	0.7	0.3	0.1	1.0	1.0	1.9
2	3	1.6	1.8	2.1	1.6	8.2	0.0	0.0
1	4	0.0	0.0	1.0	1.1	0.3	1.7	0.0
1	4	4.6	0.2	1.3	0.0	0.8	0.1	1.5
1	4	0.4	0.3	0.4	0.2	1.8	0.4	1.8
2	4	2.3	5.1	2.4	3.6	2.1	2.1	9.4
2	4	0.3	0.0	0.8	0.1	2.4	1.8	0.2
2	4	3.4	0.0	2.4	7.2	5.5	2.9	3.3

APPENDIX G
MYCORRHIZAL COLONIZATION OF ROOT TISSUE

Plot	Block	Treatment	present	absent	% Colonization	total intersects
5	1	1	113	182	38.31	295
6	1	1	91	174	34.34	265
11	1	1	110	193	36.30	303
1	2	1	167	269	38.30	436
7	2	1	99	176	36.00	275
12	2	1	131	227	36.59	358
4	1	2	216	133	61.89	349
8	1	2	170	106	61.59	276
9	1	2	147	134	52.31	281
3	2	2	175	92	65.54	267
5	2	2	239	142	62.73	381
10	2	2	175	93	65.30	268
1	1	3	102	194	34.46	296
10	1	3	94	194	32.64	288
12	1	3	105	180	36.84	285
2	2	3	120	207	36.70	327
4	2	3	170	315	35.05	485
9	2	3	87	166	34.39	253
2	1	4	179	103	63.48	282
3	1	4	198	123	61.68	321
7	1	4	171	118	59.17	289
6	2	4	183	103	63.99	286
8	2	4	204	125	62.01	329
11	2	4	181	113	61.56	294