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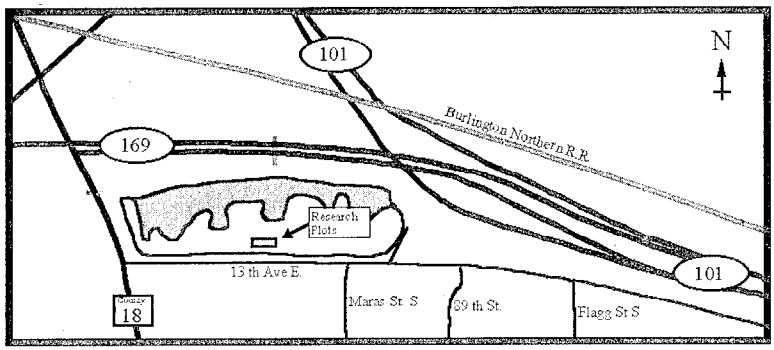

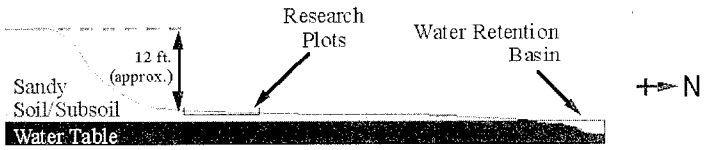

Mycorrhizal/Plant Factors Involved In Roadside Reclamation

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This research project examined mycorrhizal and plant factors involved in roadside reclamation. Research objectives included:

- Producing mycorrhizal inocula for incorporation at restoration sites
- Assessing the properties of soil additives and mycorrhizal inocula in potted greenhouse trials
- Assessing the effects of soil amendments and maintenance techniques on mycorrhizal/plant parameters on recently established roadside rights-of-way
- Assessing the longer term effects of mycorrhizal reintroduction into prairie restoration sites
- Monitoring mycorrhizal diversity of undisturbed Minnesota prairies for comparison to restoration sites
- Monitoring plant colonization in wetland and prairie habitats at a restored roadside site

Fertilization amendment favored the growth of undesirable weedy species and lowered the diversity of native plant species. Therefore, the addition of fertilizer did not benefit native prairie plantings and generally is not recommended for use at roadside prairie restoration sites. The fungal inocula incorporated into roadside restoration plots enhanced native plant cover at one location, but had minimal impact at another location. Our results indicate that many factors influence the outcome of fungal inoculation. The mycorrhizal studies of native areas provide an information base against which to compare restored areas. The revegetation studies yielded recommendations for future restorations.

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MYCORRHIZAL/PLANT FACTORS INVOLVED IN ROADSIDE RECLAMATION

Final Report

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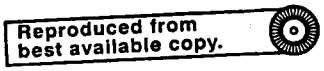

EXECUTIVE SUMMARY

The Minnesota Department of Transportation (Mn/DOT) is designating funds and research efforts towards delineating a management strategy for restoring and maintaining tallgrass prairie. Mn/DOT is responsible for the maintenance of extensive tracts of roadside grassland in Minnesota. However, little is known regarding methods for establishing native species and maintaining prairie plant communities in roadside areas. Establishing an integrated management program that promotes self-sustaining prairie vegetation is one of the most important goals in prairie research.

Many dominant tallgrass prairie plant species form symbiotic associations with arbuscular mycorrhizal (AM) fungi. These AM fungi can provide a number of benefits to their plant host including improved nutrient availability. Unfortunately, prairie restorations are often conducted on sites that have been severely disturbed and lack a viable population of mycorrhizal fungi. The lack of fungal propagules and subsequent lack of mycorrhizal symbiosis are hypothesized to result in reduced productivity of native species at restoration sites.

The main goal of this research was to study mycorrhizal and plant factors involved in roadside restoration. Our objectives included assessing the properties of mycorrhizal inocula and soil additives for their immediate and long term effects at roadside restoration sites following construction and in potted greenhouse trials. In addition, the mycorrhizal diversity of remnant prairies and methods of mycorrhizal inocula production using the fungal species from these remnant sites were assessed. Finally, a study was initiated to examine alternative maintenance techniques for roadside prairies.

Mycorrhizal inoculation of restoration sites can improve plant establishment and potentially accelerate plant succession. The Beltsville nutrient system functioned well in the production of native prairie mycorrhizal inoculum using big bluestem as a host plant. However, in-house production of local ecotype, mycorrhizal inoculum is costly and time consuming, limiting field application to small-scale endeavors. The availability of less costly commercial inoculum products can greatly increase the range of situations where addition of more universal, mycorrhizal inoculum is economically feasible. Commercially available mycorrhizal inoculum was tested for infectivity and composition.

The effects of soil amendments and maintenance techniques on mycorrhizal/plant parameters on recently established roadside rights-of-way was assessed. To ameliorate the less than ideal soil conditions at the horticultural landscaping project done along Trunk Highway 280 in the city of St. Paul, Minnesota, a number of soil amendments were added during the installation of the plants. The landscaping has shown high survival and plant growth has been has been vigorous, regardless of soil amendment treatment. Soil nutrients, especially nitrogen, phosphorus and

potassium, should be measured at each site before addition of mycorrhizal amendments. If soil nutrient content is high, addition of mycorrhizal inoculum is unlikely to be of value.

An experimental prairie restoration was established at a wet prairie site in central Minnesota. The purpose was to examine methods of increasing nutrient availability to native plants during restoration. Results supports the view that fertilization should not be used on prairie restoration under most circumstances. Futhermore, mycorrhizal amendments may benefit highly degraded restoration sites but may not be cost effective at most typical restoration sites.

Though we have suggested possible improvements of Mn/DOT's seeding methods, the success of our restoration using Mn/DOT's protocols demonstrates that the current methods work well. Precisely following the guidelines of the Minnesota Department of Transportation Seeding Manual 1996/97, we established a vigorous prairie plant community with a good balance of forbs and grasses. Our suggested improvements are given to improve the current guidelines and widen the scope of native plantings that Mn/DOT can conduct during their work.

The influence of maintenance techniques was examined along Minnesota roadside areas. Roadside restoration sites were evaluated following a burn. Additionally, some plots were mowed and the vegetation was left on site. Results indicate an inconsistent effect of treatments on vegetation, which could be due in part to differences between initial vegetation and soil characteristics between plots. Future studies should have more uniformity in vegetation composition or soil properties among blocks.

The longer-term effects of mycorrhizal reintroduction into prairie restoration sites were assessed at the upland JES restoration site. Prairie ecosystems are typified by low and generally constant nutrient availability, and many of the dominant plant community members are obligately mycorrhizal. Our earlier study at JES restoration site showed that inoculated plots had greater native planted species cover than uninoculated control plots. The longer-term effect of mycorrhizal inoculation in these plots suggest that the benefits of inoculation occur early after establishment.

The characterization of the mycorrhizal communities of native remnant prairies, for comparison to the community at restored prairies in the region was conducted. The analysis of AM fungal community composition at prairie areas using spore morphology is described.
The composition of vegetation and long-term success of the restoration efforts at the JES Restoration Prairie/Wetland Complex near Cambridge, Minnesota was assessed. The JES restoration site has a diversity of plant species in both the upland and wetland areas, many of which are indigenous desirable species. Most of the planted and/or seeded species remain present at the site. However, invasive species are a problem and recommendations are suggested for the control of these species.

Chapter 1. Introduction

Recognition of the importance of tallgrass prairies to the ecology and natural history of Minnesota has prompted increased attempts to restore prairies throughout the state. The Minnesota Department of Transportation (Mn/DOT) is one of many organizations that have begun to designate funds and research efforts towards delineating a management strategy for restoring and maintaining tallgrass prairie. Mn/DOT is responsible for the maintenance of extensive tracts of roadside grassland in Minnesota. However, little is known regarding methods for removing exotics, establishing native species, and maintaining prairie plant communities in roadside areas. Establishing a management program that promotes self-sustaining prairie vegetation is one of the most important goals in current prairie research.

Many dominant tallgrass prairie plant species form symbiotic associations with arbuscular mycorrhizal (AM) fungi by providing them with carbohydrates produced during photosynthesis. In turn, AM fungi can provide a number of benefits to their plant host, including improved nutrient availability and increased drought tolerance [1]. Unfortunately, prairie restorations are often conducted on sites that have been severely disturbed and lack a viable population of mycorrhizal fungi [2, 3]. The lack of fungal propagules and subsequent lack of mycorrhizal symbiosis are hypothesized to result in reduced productivity of native species at restoration sites.

The main goal of this research was to study mycorrhizal and plant factors involved in roadside restoration. Our objectives included (1) producing mycorrhizal inocula for incorporation at restoration sites, (2) assessing the properties of soil additives and mycorrhizal inocula in potted greenhouse trials, (3) assessing the effects of soil amendments and maintenance techniques on mycorrhizal/plant parameters on recently established roadside rights-of-way, (4) assessing the longer-term effects of mycorrhizal reintroduction into prairie restoration sites, (5) monitoring mycorrhizal diversity of undisturbed Minnesota prairies for comparison to restoration sites, and (6) monitoring plant colonization in wetland and prairie habitats at a restored roadside site.

Chapter 2, a study of mycorrhizal inoculum production for incorporation at restoration sites, addresses objective 1. A demand for large quantities of arbuscular mycorrhizal (AM) inocula has been created by the recent interest in mycorrhizal inoculum application at revegetation sites. Restorationists and others, concerned about potential problems resulting from

application of non-native fungal species have resisted the use of non-local AM fungal ecotypes found in commercial inocula. As an alternative, some mycorrhizal researchers produce their own inoculum from regional AM species [4, 5]. The goal of this study was to determine whether the Beltsville nutrient system could be used to produce native prairie mycorrhizal inoculum.

Chapter 3, assessing the properties of soil additives and mycorrhizal inocula in potted greenhouse trials, addresses objective 2. Mycorrhizal inoculation of restoration/reclamation sites can improve plant establishment, and potentially accelerate plant succession [5, 6, 7]. However, in-house production of mycorrhizal inoculum is costly and time consuming, limiting field application to small-scale endeavors. The availability of less costly commercial inoculum products can greatly increase the range of situations where addition of mycorrhizal inoculum is economically feasible. This study tested the infectivity and composition of commercially available mycorrhizal inoculum. We conducted two experiments, one focusing on ectomycorrhizae, and one focusing on arbuscular mycorrhizae.

Assessing the effects of soil amendments and maintenance techniques on mycorrhizal/plant parameters on recently established roadside rights-of-way is objective 3. We selected four sites for assessment. Chapter 4 deals with a site along Trunk Highway 280 in the city of St. Paul, Minnesota. Chapter 5 discusses the experimental prairie restoration we established at a wet prairie site in central Minnesota. And finally, Chapter 6 addresses the influence of maintenance techniques (burning, mowing) on the vegetation, soil properties and AM colonization of plants along Minnesota roadside areas.

Chapter 4 addresses the horticultural landscaping project done along Trunk Highway 280. In 1996, Mn/DOT completed a resurfacing and rebuilding project for TH280, which greatly disturbed the soil present along TH280. To ameliorate the less than ideal soil conditions, a number of soil amendments were added during the installation of the plants. Transplants received one of five amendment treatments, or remained as unamended controls. The purpose of this study was to determine whether the amendments improved plant establishment and growth. Two approaches were taken. First, overall survival and health were estimated across all planted species to see if there were broad treatment effects. Second, in depth analyses were performed for three species groups: the sumacs, the roses, and the oaks.

Chapter 5 addresses the experimental prairie restoration we established at a wet prairie site in central Minnesota to examine methods of increasing nutrient availability to native plants

during restoration. Several mycorrhizal and fertilizer amendments were combined with two application techniques during establishment of the restored prairie. Mycorrhizal inoculum amendments were selected based on their availability to researchers and restorationists. Fertilizer treatments were chosen to represent methods and rates of fertilization currently used by vegetation managers [8]. In conjunction with Mn/DOT's seeding methods, we examined the effect of mycorrhizal and fertilizer amendments on the plant community. Our suggested improvements to Mn/DOT's seeding methods are given to enhance the current guidelines and widen the scope of native plantings that Mn/DOT can conduct.

Chapter 6 concerns the influence of maintenance techniques (burning, mowing) on mycorrhizal colonization and the prairie plant community. The success of the diverse plants within the tallgrass prairie community was supported largely through the action of periodic fires. However, recently fire has been excluded from most prairie areas; consequently, many of the existing grassland areas are now dominated by weedy or exotic plant species.

Research suggests that mowing can have a similar effect as burning on the prairie plant community [9, 10, 11, 12]. Mowing of roadside areas has been done extensively in the past; however, the exact impact of annual mowing on restored prairies is still unclear. It is possible that mowing along roadside areas could reduce cover of exotic species and promote increased cover and diversity of native vegetation.

The goal of this research was to monitor the effect of burning and mowing on the vegetation and soil parameters in roadside areas in Minnesota. Roadside restoration sites in St. Cloud and Cambridge were evaluated following a burn. Additionally, some plots in St. Cloud were mowed and the vegetation was left on site. This treatment mimics one of the many possible mowing treatments that has been used by Mn/DOT to control the vegetation in roadsides. We hoped to determine if burning is an effective strategy for creating and maintaining native plant populations while at the same time decreasing the cover of unwanted exotic plant species.

Objective 4 is addressed in Chapter 7, assessing the longer-term effects of mycorrhizal reintroduction into prairie restoration sites. Prairie ecosystems are typified by low nutrient availability, and many of the dominant plant community members are obligately mycorrhizal. It has been hypothesized that inoculation would promote growth of obligately mycorrhizal late-successional species over ruderal, early-successional species that are often non-mycorrhizal.

This hypothesis was substantiated by a field experiment by Smith et al. [5]. This study showed that mycorrhizal inoculation successfully increased mycorrhizal activity under field conditions and that inoculated plots had greater native planted species cover than uninoculated control plots. Chapter 7 reports on the longer-term effects of mycorrhizal inoculation in these plots, which have been monitored for five years. Given the expense and effort involved with the inoculation process, it is important to document whether long-term gain is achieved through mycorrhizal inoculation.

Chapter 8 addresses the monitoring of mycorrhizal diversity of undisturbed Minnesota prairies for comparison to restoration sites. This is objective 5. The purpose of Chapter 8 is to characterize the mycorrhizal communities of native remnant prairies, for comparison to the community at restored prairies in the region. Our eventual goal is to determine whether a comparable mycorrhizal community is developing in the restored sites. This chapter describes the analysis of AM fungal community composition at remnant prairie areas using spore morphology.

Chapter 9 addresses objective 6 by documenting the composition of vegetation and long-term success of the restoration efforts at the JES Restoration Prairie/Wetland Complex near Cambridge, Minnesota. Three approaches were taken. First, a walk through inventory of plant species composition was conducted, for comparison of extant species with species seeded and planted at the site. Second, permanent vegetation plots were set up, and species composition in 1996 versus 1999 was compared. Third, the composition of the seed bank was examined and compared to existing vegetation by Marcia Raley for part of her MS thesis research project at the University of Minnesota. From these studies, it became clear that invasive species are a problem at this site, and recommendations are suggested for the control of these species.

Chapter 2. Production of Minnesota native prairie arbuscular mycorrhizal inoculum

2.1 Overview

A demand for large quantities of arbuscular mycorrhizal (AM) inocula has been created by the recent interest in mycorrhizal inoculum application at revegetation sites. However, indigenous inocula are not commercially available for specific ecological habitats or vegetation types. This is especially important to restorationists and others concerned about potential problems resulting from application of non-native fungal species. Hence, they have resisted the use of non-local inocula ecotypes found in commercial inocula. As an alternative, some mycorrhizal researchers produce their own inoculum from regional AM species [4, 5].

Unfortunately, the high cost of producing prairie AM inoculants is one of the main reason that few restoration studies have been conducted to examine the ability of mycorrhizal amendments to improve restoration plant communities. Unlike other soil amendments, mycorrhizal fungi and fungal inoculants are living organisms and must be carefully cultured and maintained over a period of months with suitable host plants. The time and resources required in production of inocula are considerable and have made inoculum production very costly. Our work was conducted with the goal of producing native prairie AM inoculum and examining less costly methods of inoculum production.

Although arbuscular mycorrhizal fungal inocula can be produced by several methods, all utilize the same principles to produce inocula. The generic method is to culture small soil samples, which contain AM fungi collected from remnant sites, in larger quantities of sterile soil [4]. Culturing containers are seeded with suitable host plants for the AM fungal species and the plants are grown to maturity, allowing mycorrhizal fungi to colonize the roots and produce spores. Finally, the pots are left unwatered to senesce and thoroughly dry. Soil in the pots can be used as inoculum after a brief cold treatment to break the dormancy of certain fungal spore species. The soil will contain a mixture of mycorrhizal spores, fungal hyphae and root pieces colonized by mycorrhizae, which are all thought to be effective in inoculation [1].

Using this methodology, a total of 207 mycorrhizal propagation pot cultures were carried out from 1997 to 1999 under a variety of conditions (growth chamber versus greenhouse, automatic watering system versus hand watered, different host species, different growth periods, etc.). A total yield of approximately 250 kg of mycorrhizal inoculum was obtained for future

use. A majority of these pot cultures used soil that originated from native prairie and wetland sites, and would be appropriate for use as native inoculum (Table 2.1). These cultures also serve as a means for identification and characterization of the mycorrhizal communities of native areas, which will be discussed in Chapter 8.

Table 2.1. Pot culture mycorrhizal propagation, 1997-1999.

Pot culture soil source	Number of pots of inoculum produced
Natural areas	
Crosstown Prairie	106
Feder Prairie	11
Helen Allison Prairie/Savanna	18
Schaefer Prairie	6
Country Club Wetland	19
Restored areas	
JES upland prairie	18
JES wetland	4
Shakopee	1
Other	
Commercial inoculum	24
Total	207

2.1.1 *Arbuscular mycorrhizal inoculum production using the Beltsville nutrient watering system*

Research production methods for AM inoculum have provided researchers with only enough inoculum to treat a number of small research plots. An estimated application rate for a large study area would be approximately 1 ton of inoculum per hectare, though the inoculum application rates varies greatly in agricultural and restoration literature [4, 13, 14]. Typical inoculum production methods would not supply enough inoculum to apply inoculum to 1 acre. Providing a larger inoculum supply could allow further study of mycorrhizal amendment use by researchers and vegetation managers. In this experiment, we examined the Beltsville inoculum production system as a less costly and less labor intensive method of producing high quality native prairie AM inoculum.

The Beltsville system is a hydroponic method of culturing AM fungi and their host plants, which uses silica-sand as a vehicle to distribute a plant nutrient solution [15]. With this system, Millner and Kitt found that AM spore production was very high with a minimal input of

time and equipment. Their results suggested to us that the Beltsville system would have great potential for production of native prairie AM inocula.

The goal of this study was to determine whether the Beltsville nutrient watering system would suit our needs for production of native prairie mycorrhizal inoculum. Of primary importance was the amount and quality of inoculum that could be produced using our facilities. Large amounts of inocula containing high concentrations of propagating spores, hyphae, and colonized root pieces are desired for their ability to provide many fungal propagules when applied [1].

A second aim of our work was to assess the use of a native plant species as a host for mycorrhizal fungi under the Beltsville culturing system. Commonly, mycorrhizal culturing techniques use agronomic plants such as maize or beans. A native prairie host was tested with the idea that it would best foster the reproduction of native prairie AM propagules. Big bluestem (*Andropogon gerardii* Vitman) was selected because it is a hardy obligate mycorrhizal species commonly found in prairies [16].

The third objective was to examine the efficiency of a liquid spore AM inoculum. A liquid spore suspension could be easily applied to a large-scale restoration with a small hand sprayer. Storage of a concentrated liquid inoculum would also be more convenient than bulky soil inoculum.

2.2 Materials and Methods

2.2.1 *Inoculum*

Soil and spore inocula were generated in previous lab studies using soil from the Crosstown remnant prairie soil site, located near Minneapolis, MN [17]. The first inoculum treatment was a soil-only treatment using 51.7 g of the Crosstown inoculum. Secondly, we used a liquid suspension treatment of Crosstown prairie spores. Spores were isolated by the sucrose density centrifugation method [18], suspended in water, and stored at 4° C until use. Immediately prior to use, the spore suspension was diluted to yield approximately 500 spores per 5-ml treatment dosage. All spore suspensions were applied less than 48 hr after the spores were isolated from soil. A third combination inoculum treatment containing both 51.7 g of soil and 5 ml of spore suspension was also used. Controls for soil inoculum and spore suspension were prepared by autoclaving the soil and spore suspensions (121°C 20 lbs. psi) for 20 min.

2.2.2 *Potting and inoculation*

Black plastic pots, 14.5 cm in diameter, were filled 11 cm deep with moist uniformly graded silica-sand minispheres (Unimin corp, Le Suer, Mn). Pots receiving the spore suspension treatments had 2 ml of spores added directly to the sand surface, seeds added, then the remaining 3 ml of spores were applied. Soil treatment pots had 51.7 g of soil added to the surface of the sand, followed by a layer of seeds. The combination treatment of soil and liquid spore inoculum was applied in the same fashion as the individual treatments. Seeds for all treatments were added by weight, with approximately 348 imbibed and surface-sterilized big bluestem seeds in each pot. Seeds were covered with a layer (1.5-cm) of silica-sand and drip irrigation watering rings were placed on top of the sand. Pots were temporarily (4 days) covered with plastic to prevent drying and contamination. Fifteen pots were established for each treatment. Five pots were prepared as controls for each treatment using sterilized inoculum.

2.2.3 *Beltsville equipment*

The Beltsville system is an irrigation system constructed from easily available electronic, horticultural, and household supplies (Figure 2.1) [15]. The main electric pump delivers nutrient solution from a storage tank to individual pots through the main supply line. Each pot contains a drip line, which is sized to regulate the flow of solution from the supply line to the pot. The pump, activated according to a user-entered program, is controlled by an electronic timer. The amount of nutrients provided to the pots is determined by the duration of each on-off cycle of the supply pump. A secondary recirculating pump is used to mix the contents of the storage reservoir while the supply pump is in operation.

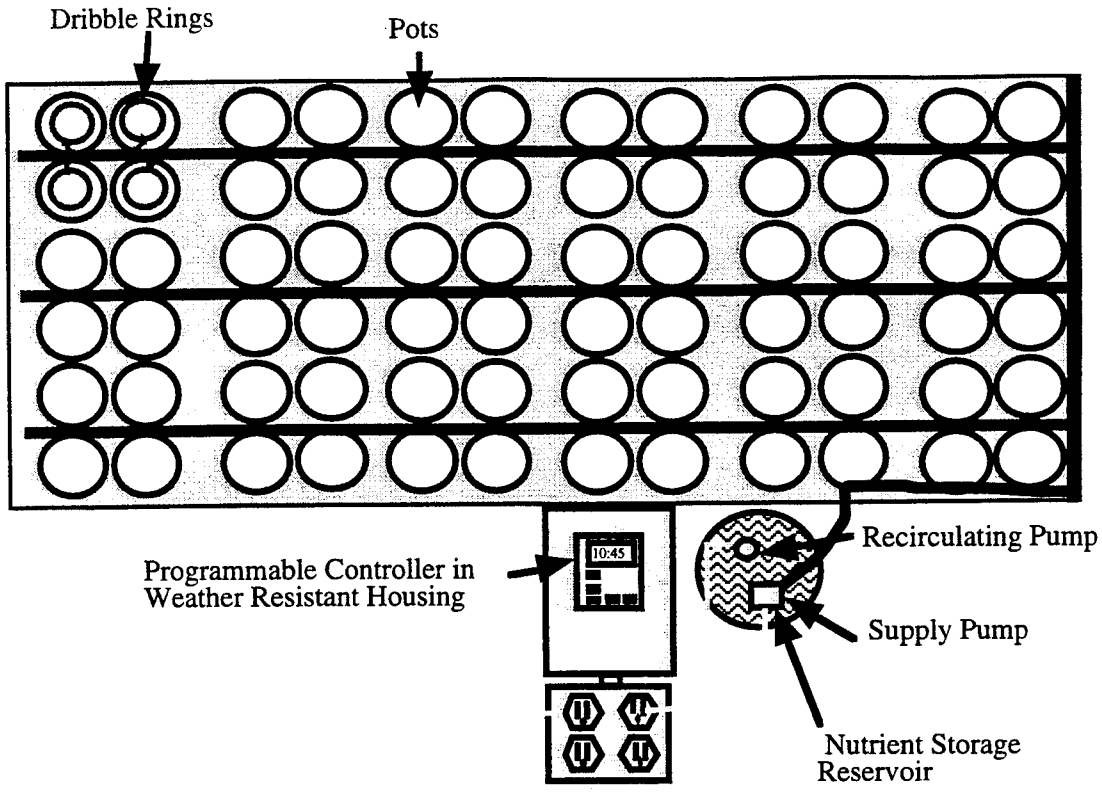

Figure 2.1. Layout of Beltsville automatic plant water/nutrient delivery system for production of arbuscular mycorrhizae inoculum. Layout will vary as water supply lines can be added to allow many configurations of pots.

2.2.4 *Cultivation*

Pots were placed in the University of Minnesota, College of Biological Science Greenhouse and irrigated 5 times daily with approximately 65 ml of solution. Initially plants were watered with tap water, following emergence a modified half-strength Hoagland's nutrient solution (Table 5.2) was applied [19]. All plants were watered from the same nutrient reservoir. Phosphorus was given in excess during the first 2 weeks of growth, then limited to 10 μM. Fresh nutrient solution was added to a storage tank at approximately 3-day intervals throughout the experiment. After 14 wk of growth, an additional 30 mls of solution was applied to the plants during each watering. Plants were maintained at ambient greenhouse temperatures from August 5, 1996 until Jan 16, 1997 (16 wk). During this period, average daily soil temperature fluctuated from 16 to 32 °C (60 to 90 °F), with a peak of 41 °C (105 °F) and low of 13 °C (55 °F). In addition to naturally occurring light, artificial light was continuously applied.

Table 2.2. Modified Hoagland's solution

Compound	Concentration
$Ca(NO_3)_2*4H_2O$	2.5 mM
KNO_3	2.5 mM
$MgSO_4*7H_2O$	1.0 mM
NaFe EDTA (H_20)	0.05 mM
Micronutrient Stock	
$CuSO_4*5H_2O$	0.5 µM
$CoCl_2*6H_2O$	0.2 µM
$NiSO_4*6H_2O$	0.2 µM
H_3BO_3	10.0 µM
$MnCl_2*4H_2O$	2.0 µM
$ZnSO_4*7H_2O$	1.0 µM
$NaMoO_4*2H_2O$	0.2 µM
HCL 3N	As needed
KOH	As needed
KH_2PO_4	10 µM
MES buffer	0.5 µM

2.2.5 *Harvesting*

During harvesting, plants were scored for symptoms of phosphorus deficiency. Scoring was visually assessed on a 1 to 5 scale indicating the amount of anthocyanin pigmentation, which is a dark red-purple discoloration of leaves common in plants grown under low phosphorus conditions [20]. Stems were removed from all plants, placed in separate paper bags and dried for biomass analysis. All control plants and 3 pots of each treatment were used for root biomass and colonization determination. Pots selected for root analyses were carefully emptied of the root mass and sand. One half of the root mass was cleared of sand and put in a 65 °C (149 °F) drying oven for at least 21 days, while the other half was rinsed clean and stored in 50% ethanol for later mycorrhizal colonization analysis. All pots not used for root examination were returned to the greenhouse and allowed to senesce until dry (approximately 6 weeks). After drying, the soil and roots from the pots were placed individually in plastic containers and stored at 4 °C (39 °F).

2.2.6 *Spore isolation*

Spore isolation was performed on treatment pots not used for root studies. Fifteen grams of soil were wet sieved to collect material between 250 and 38μm in size. Sieved material (containing mycorrhizal spores) was further purified by sucrose density centrifugation which seperates spores from unwanted debris [18]. Spores were placed on grided filters and counted under a dissecting scope at approximately 60× magnification.

2.2.7 *Root colonization*

Roots stored in 50% ethanol were cleared for 7.5 min. in 10% KOH at 90° C then acidified with 1% HCl for 1 hr at 90 °C (195 °F) and stained with trypan blue. Roots were destained in acidic glycerol and stored at room temperature until analysis. Roots from each sample were cut into approximately 1-cm pieces and random subsamples were permanently mounted on microscope slides. Percent colonization was evaluated using the magnified intersection method at 100-400× magnification [21].

2.3 Results

Seeds began germinating within 5 days of planting and by 21 days all pots had a dense foliage layer. The germination rate was lower than expected based on PLS (pure live seed) information provided by the seed producer; however, the seeds that did germinate quickly formed a barrier to limited soil surface contaminants.

Plants in pots treated with either the liquid spore suspension or control treatments exhibited anthocyanin pigmentation indicative of phosphorus deficiency. These symptoms appeared within 3 weeks of lowering the phosphorus levels and continued until the end of the experiment (Figure 2.2). Plants inoculated with soil or the combination treatment did not exhibit phosphorus deficiency symptoms and were greener and fuller throughout the experiment.

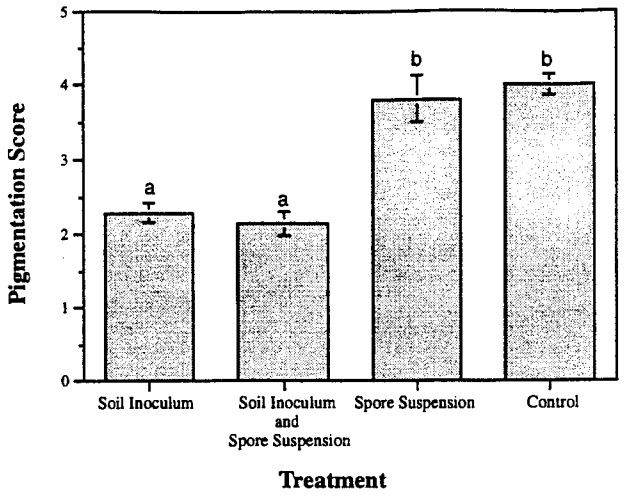

Figure 2.2. Mean anthocyanin pigmentation ± 1 SE. Higher numbers represent more anthocyanin pigmentation. Different letters indicate significant differences between treatments ($p < 0.05$)

Plants receiving the liquid spore suspension or control treatments were slightly taller (Figure 2.3) and had more above ground biomass (Figure 2.4) than plants treated with soil inoculum or the combination treatments. They also were more advanced in floral development with several pots having mature seeds by the conclusion of the experiment, as compared to the soil and combination treatment plants, which had no signs of floral development. Root mass differences between treatments were not found to be significant.

Root AM colonization levels in the soil and combination inoculum treatment pots were substantial (Figure 2.5). In contrast, plants treated only with the spore suspension had few colonized roots. Colonization in pots treated with either liquid spore suspension or control pots was not significantly different from zero.

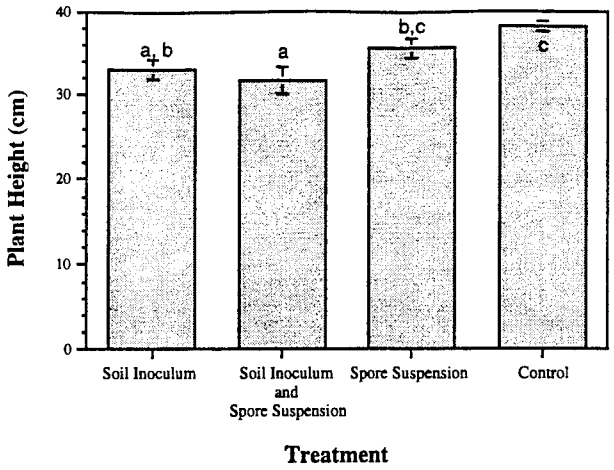

Figure 2.3. Mean (±1 SE) plant height. Different letters indicate significant differences among treatments ($p < 0.05$)

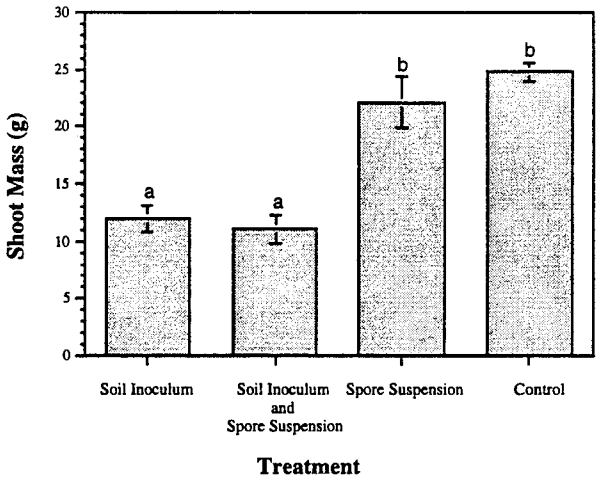

Figure 2.4. Mean (± 1 SE) dry mass of plant shoots. Different letters indicate significant differences among treatments ($p < 0.05$).

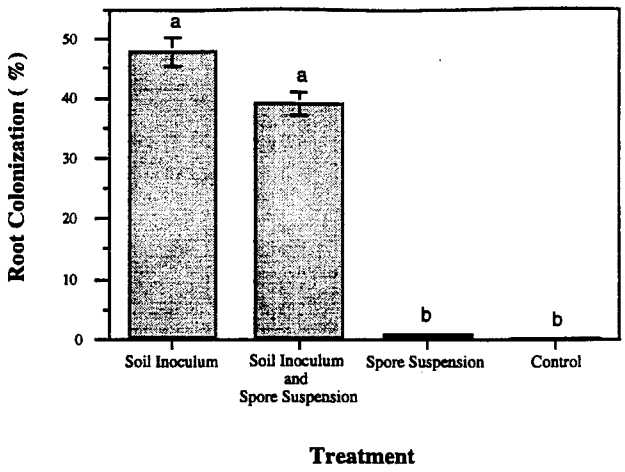

Figure 2.5. Mean (± 1 SE) AM colonization levels. Different letters indicate significant differences among treatments ($p < 0.05$).

Although fungal colonization was present in the soil and combination treatments, few mycorrhizal spores were found in any of the treatments (Figure 2.6). However, the soil and combination treatments had significantly more spores per gram of soil than did either the liquid suspension or control treatment. Identification of spores from the treatment pots revealed spores of these species; *Glomus mosseae*, *Glomus occultum*, and *Glomus etunicatum*. Immature spores of other species also may have been present.

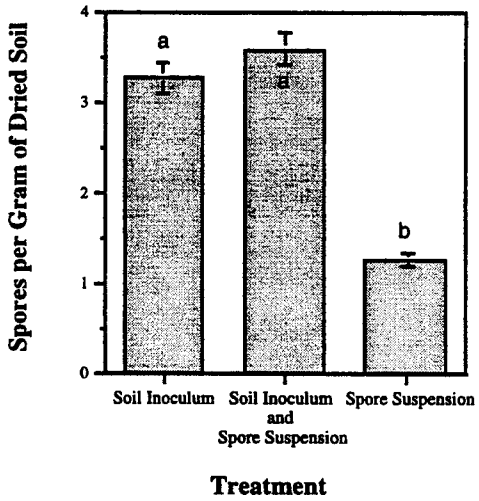

Figure 2.6. Mean (± 1 SE) spore number per gram of dried soil. Different letters indicate significant differences among treatments ($p < 0.05$).

2.4 Discussion

Big bluestem appears to be very amenable to growth in sand-microspheres. Plants grown via the Beltsville method grew to an average height of 36 cm, nearly twice the height of big bluestem plants previously grown in our inoculum production using alternate methods [22]. Excellent growth may indicate that less concentrated nutrient solution should be used. The lack of colonization in these treatments (Figure 2.5) in conjunction with significant growth of our plants (Figure 2.3) indicates that the AM dependent relationship of big bluestem found in plants potted in prairie soils did not hold in our nutrient pot culture system [16]. The normal dependency of big bluestem on mycorrhizal fungi might be unnecessary for the plant should the nutrient solution be too rich. It might therefore be necessary to decrease the concentration of the nutrient solution so that big bluestem grown using this method is more dependent on mycorrhizae and more readily supports growth and reproduction of these beneficial fungi.

The high AM fungal colonization of plants treated with soil inoculum and combination treatments indicated that mycorrhizal fungi were present (Figure 2.5) yet spore numbers were relatively low (Figure 2.6). Several possible reasons exist for low spore numbers. A likely explanation is that spores were consumed by nematodes which, based on our observation during spore quantification, may have been present in large numbers during the experiment. Nematodes are microscopic threadworms known to eat AM fungal spores. Nematodes are likely to have been present in the greenhouse or could have entered through the greenhouse ventilation system, which is next to heavily tilled agricultural fields. Another possible source of nematode contamination was the soil used to inoculate the pots, which was originally collected at a field site. Nematode contamination of inoculum led us to use cleaner environmental growth chambers in later studies.

Another factor that likely hampered inoculum production was the high greenhouse temperatures during early fall. Soil temperatures were commonly 100 °F (37.7 °C) and a peak temperature of 105 °F (40.5 °C) was recorded on a particularly warm day. Heat damage also occurred to a few pots during a weed-control 'burn' conducted by greenhouse staff with a propane blowtorch. Use of well-regulated environmental growth chamber will eliminate these extreme temperatures.

Another finding was that a liquid spore suspension did not function as effectively as soil inoculum in our system. Application of a liquid spore inoculum has been tested by other

researchers with success [13], which indicates that our spore suspension may have had a low number of propagating spores. Based on AM spore counts of the soil inoculum obtained after beginning the experiment, our suspension likely did have fewer spores than was found in the soil inoculum. Another potential problem may have been that the liquid suspension was washed out of the pots before root colonization could occur. The large textured silica-sand microspheres provided an easy path for liquids to flow through the pot, while larger soil and root pieces in the soil inoculum could have remained in place and colonized the roots. Further work using various spore suspensions concentration and soil particle sizes may resolve the difficulties we found using the liquid spore suspension.

2.5 Conclusions

Much information can be gained from this experiment. The construction and maintenance of the Beltsville equipment was simple and quick. Though setup of inoculum culturing pots and plants was time consuming, the system ran well with very little day-to-day monitoring and adjustment. Moreover, most of the work performed during this study was to collect scientific data about the watering system itself, and should not be necessary once the optimal culturing conditions are determined. However, an issue that needs to be addressed is the maintenance of an uncontaminated environment for culturing AM fungi and the costs associated with maintaining the clean culturing environment.

The Beltsville system will work well to produce native prairie mycorrhizal inoculum with big bluestem as its host. However, more work needs to be conducted to improve the number of mycorrhizal spores present. The level of colonization suggests that the inoculum has a significant number of mycorrhizal propagules, yet an enhanced spore component would likely improve inoculum longevity as spores are thought to be the longest lived of mycorrhizal propagules [1].

A final observation is that this method produces inoculum with a very good texture. The large grained, dust-free sand gave the inoculum properties that proved invaluable during spore isolations conducted on the inoculum. Typically, soil based inocula require a great deal of wet sieving during spore isolation. Inoculum produced via the Beltsville system required almost no sieving and was therefore easier to work with. These same properties may indicate that

Beltsville produced inoculum may work well with current seeding and fertilization regimes during application at restoration sites.

2.6 Recommendations

1.) The Beltsville system appears to function very well in the production of native mycorrhizal inoculum using big bluestem as a host plant. It produces large quantities of inoculum with a minimum of cost and effort. However, before large batches of mycorrhizal inoculum are produced, minor adjustments should be made to the system to optimize inoculum production. The adjustments could include the following:

   a) Assessing different nutrient levels to improve production of prairie inoculum using the Beltsville AM culturing system.

   b) Use of a cleaner environment to eliminate potential contamination and pest problems.

   c) Altering native plant host species or use multiple species to determine which species are capable of producing inoculum with the highest inoculum potential.

2) Further research using a combination of native plants could be useful in producing an inoculum with the widest possible number of mycorrhizal fungal species.

3) Hydroseeding of soil and/or liquid inocula may be an effective application method for mycorrhizal fungi and the potential use of hydroseeding for inocula and native plants warrants future research.

Chapter 3. Commercial inoculum pot culture studies

3.1 Overview

Mycorrhizal inoculation of restoration/reclamation sites can improve plant establishment, and potentially accelerate plant succession [5, 6, 7]. However, in-house production of mycorrhizal inoculum is costly and time consuming, limiting field application to small-scale endeavors. The availability of less costly commercial inoculum products can greatly increase the range of situations where addition of mycorrhizal inoculum is economically feasible.

It is unclear, however, that commercial inoculum will be effective under local conditions. The centralized production of such inoculum means that the site of mycorrhizal origin is usually geographically distant from the site of product application. The extent of ecotype variation in mycorrhizal fungi is not known, but there is ample evidence that fungal species and ecotypes are adapted to local edaphic conditions such as soil pH and nutrient availability [23, 24], and can differ in their effectiveness as plant mutualists [25, 26]. Moreover, ectomycorrhizal species are often highly host specific, making it difficult to produce an inoculum that might be effective with a wide variety of host species. To overcome these limitations, commercial production has apparently focused on selecting fungal strains that thrive under a wide range of conditions and host species [27]. That they have succeeded in this task has not been sufficiently tested, however.

The purpose of this study is to test the infectivity and composition of commercially available mycorrhizal inoculum. We conducted two experiments, one focusing on ectomycorrhizae, and one focusing on arbuscular mycorrhizae.

3.2 Ectomycorrhizal study

To test the effectiveness of commercially available ectomycorrhizal products, an experiment was set up using bur oak (*Quercus macrocarpa*). Bur oak was chosen for this study because it is a common Minnesota species that forms ectomycorrhizal associations, and is frequently used in Mn/DOT roadside plantings. For example, bur oaks were planted in 1997 along Trunk Highway 280 in St. Paul, MN, after road construction. Many of these trees received a commercial inoculum product, MycorrTree™ Treesaver (Plant Health Care, Inc., Pittsburgh, PA), at the time of planting to aid tree establishment and improve tree vigor. The MycorrTree

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product contains an ectomycorrhizal species, *Pisolithus tinctorius*, which has been characterized as a generalist, and has been documented to colonize at least 50 tree species, including bur oak [27].

3.2.1 *Materials and methods*

Prior to initiating the experiment, acorns were gathered from a single oak tree in August 1998 and stored in damp vermiculite at 4 °C for one month. Acorns were then surface sterilized in a dilute bleach solution and planted in trays of pasteurized soil. This soil originated from an unamended area of the Mn/DOT TH280 planting, and was steam sterilized three times prior to use. The trays were covered with plastic wrap to maintain high humidity for germination.

Sixty germinated seedlings were individually transplanted into separate 5 1/2 inch pots, receiving one of six possible treatments (Table 3.1). Oaks in treatments 1 and 2 were grown in sterilized field soil with old or new batches of MycorrTree commercial inoculum product, respectively. The old batch corresponds exactly to the inoculum product used at the TH280 planting, and was stored at 4 °C for approximately 1year. The new inoculum was purchased immediately prior to this experiment, and differed slightly in formulation from the original batch of inoculum. Treatments 3 & 4 were controls to test for inadequate soil sterilization and greenhouse contamination, respectively. By planting oaks in nonsterile field soil without inoculum, treatment 5 tested whether oaks could be colonized by existing mycorrhizal propagules found at the site. If so, treatment 6 would then indicate whether commercial inoculum resulted in increased colonization above and beyond that produced by native soil. For all treatments that received inoculum, the inoculum was intermixed with the appropriate soil before planting the oak seedling. In all treatments except #4, the soil was covered with foil with the seedling protruding through. The seedlings were watered three times a week, and allowed to grow in the greenhouse for 5 months.

Table 3.1. Treatments used in ectomycorrhizal inoculum study.

Treatment	Soil sterilized?	Inoculum source?	Soil surface covered?
1	Yes	Old inoculum	Yes
2	Yes	New inoculum	Yes
3	Yes	No inoculum	Yes
4	Yes	No inoculum	No
5	No	No inoculum	Yes
6	No	New inoculum	Yes

In March 1999, the oak seedlings were harvested. The above-ground portion of each seedling was cut off, and the pots (containing the tree roots) were placed in plastic bags and stored at 4 °C until ready to process. To process, the root system and soil were removed from the pot. The loose soil was gently shaken out of the roots and the roots were then placed on a screen and washed with a fine spray of water to remove the remainder of the soil. One hundred root tips from each pot were examined under a stereoscope at 50× magnification to determine if mycorrhizae were present. Physical features that could be seen with a stereoscope or compound microscope were used to describe the mycorrhizae, based on the terminology of Durrall et al. [28]. The features identified in this study were: color, hyphae, branching characteristics, texture, luster, mantle, cystidia, and rhizomorph. Percent mycorrhizal colonization was compared statistically among treatments using ANOVA, followed by post-hoc comparisons using Tukey's HSD at $\alpha = 0.05$.

3.2.2 *Results*

Three mycorrhizal morphotypes were seen in the samples. Morphotype A had emanating hyphae that were septate but no clamp connections were seen. The overall appearance was woolly. The mantle was net prosynchema to interlocking irregular synechyma. The color of the mycorrhiza was white. No branching of the mycorrhiza was seen, nor were there rhizomorphs. Morphotype A was found on one seedling of treatment 1, two seedlings in treatment 2, and one seedling in treatment 4 (Table 3.2). Morphotype B had few emanating hyphae. The hyphae that were present had septa but no clamp connections. The overall appearance of morphotype B was smooth to fine grainy. The mantle was regular synenchyma, which looks like irregularly shaped cells with straight side walls. The color ranged from light tan to brown. There was some branching of the mycorrhiza, but no rhizomorphs were seen. Morphotype B was found in all ten

pots of treatment 5, and 8 out of nine pots in treatment 6. Morphotype D was similar in appearance to morphotype A, except that it had clamp connections and was thicker and grayer than A. It was seen at a low level in only in a single pot of treatment 3.

Table 3.2. Experimental EM colonization of oak seedlings in the greenhouse.

Treatment	Soil sterilized?	Inoculum source?	Number of surviving seedlings	Number of seedlings colonized	Ectomycorrhizal morphotype
1	Yes	Old inoculum	10	1	A
2	Yes	New inoculum	10	2	A
3	Yes	No inoculum	7	1	D
4	Yes	No inoculum	10	1	A
5	No	No inoculum	10	10	B
6	No	New inoculum	9	8	B

Percent mycorrhizal colonization differed significantly among the treatments. Colonization was more than 10 times higher in treatments 5 and 6 (those that received unsterilized field soil) than the other four treatments (Figure 3.1). However, treatment 6, which received commercial inoculum, did not show higher levels of colonization than uninoculated treatment 5. Similarly, inoculated treatments 1 and 2 showed no significant difference in colonization from uninoculated control treatments 3 and 4.

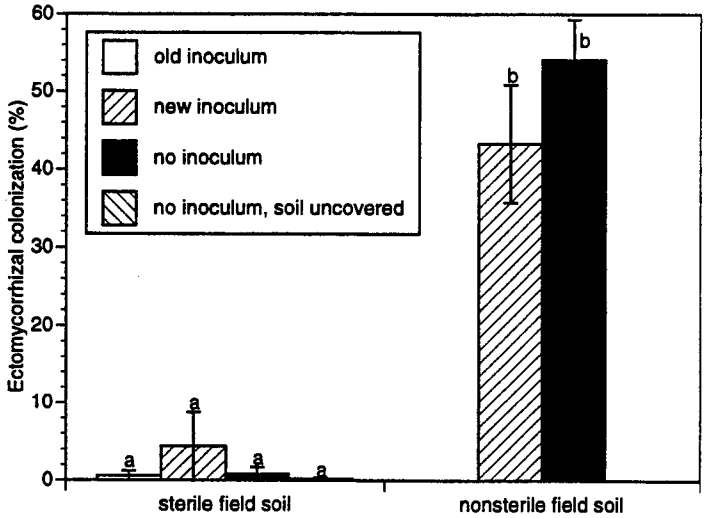

Figure 3.1. Ectomycorrhizal colonization (± 1 SE) of oak seedlings grown with or without inoculum in field soil from TH280.

3.2.3 *Discussion*

The MycorrTree commercial product was ineffective as an inoculant under these experimental conditions. Both the number of seedlings colonized and percent root tips colonized per seedling were much greater in the pots that received unsterilized field soil than pots that received MycorrTree. The ectomycorrhizal morphotype found in the non-sterile treatments, morphotype B, was only found in these nonsterile treatments, supporting its origination from the field soil, rather than from the inoculum or greenhouse contamination. Despite the disturbed nature of this field soil from a roadside planting, sufficient mycorrhizal propagules existed for substantial colonization of bur oak. Any disturbance reduces the level of mycorrhizae in the soil [29], but it is possible that at TH280, close proximity to undisturbed soil as a natural source of inoculum, or a relatively short duration for topsoil stockpiling may have allowed reestablishment of native mycorrhizae by natural means [30].

Moreover, morphotype A, which was found in a few of the commercial inoculum pots, bears little resemblance to *Pisolithus tinctorius*, the species that is supposed to be in the inoculum. As described by Weiss [31], *P. tinctorius* hyphae have clamp connections, denoting that it is a Basidiomycete, whereas clamp connection were not observed in morphotype A. Rhizomorphs are present in *P. tinctorius*, but were not observed in morphotype A. The overall appearance of the *P. tinctorius* is felty (appressed hyphal strands, like coarsely felted wool), with a net synechyma mantle that appears under a compound microscope as close-packed hyphae covering the root surface and tip. The overall appearance of morphotype A is woolly, and the mantle is net prosynchema to interlocking irregular synechyma, which is looser hyphae over a mantle that looks somewhat like a jigsaw puzzle. The color of *P. tinctorius* is supposed to be white with a slight purple tint and darker brown spots, whereas morphotype A is simply white. Point for point, morphotype A differs from the description of *P. tinctorius*, making it unlikely that they are the same mycorrhizal species. In fact, it seems unlikely that morphotype A originated with the inoculum, given its presence in one of the uninoculated control pots. It seems more probable that morphotype A is a contaminant.

The question is, why was *P. tinctorius* colonization of bur oak not observed? One possibility is that the MycorrTree commercial inoculum product was simply not viable. Extended periods of storage reduce the infective potential of mycorrhizal inoculum [27], and even though our "new" inoculum was purchased immediately prior to use, its production date

may have been substantially earlier than the purchase date. Alternatively, *P. tinctorius* may have been unable to colonize under the culture conditions of our experiment. In general, colonization by *P. tinctorius* seems to be encouraged by the higher temperatures of more southern climates, so it is possible that our growth conditions were not optimal [32, 33, 34]. However, Marx et al. [27] described successful *P. tinctorius* colonization of bur oak in a North Dakota greenhouse, under conditions similar to our own. A more likely explanation may be that the soil pH in our experiment was inhibitory to the growth of *P. tinctorius*. According to Marx et al., *P. tinctorius* inoculum must be acidic to be effective, and the neutral to basic pH of the soil from TH280 may have negatively influenced the infective ability of this mycorrhiza.

Despite the absence of *P. tinctorius* in our laboratory experiment, the possibility remains that this mycorrhizal species was an effective colonizer under field conditions at TH280. However, the presence of morphotype B propagules in the field soil probably means that the commercial inoculum product was unnecessary at this restoration site. It is not clear how these EM species might differ in the benefit they provide to their hosts, but this could be an important topic for further study.

3.3 Arbuscular mycorrhizal examination

Work with the AM components of a commercial mycorrhizal inoculum was performed to obtain basic information about the product. Our main objectives were to determine how many viable mycorrhizal spores were present in the AM inoculum product and which species of AM fungal spores were present. MycorrTree TreeSaver was selected for analysis as it was applied in field trials at the Trunk Highway 280 site (chapter 4).

3.3.1 *Materials and methods*

Commercial AM inoculum

MycorrTree TreeSaver (Plant Health Care Inc.) is suggested for use in growth of trees and shrubs. Ingredients listed in Mycorrtree include a formulation of several biotic and abiotic components, including both endo- and ecto-mycorrhizal fungi, TerraSorb™ (a gelling agent), yucca (a wetting agent), seaweed (nutrient and growth stimulant), and humic extracts (soil conditioner and biostimulant).

In vitro spore reproduction

To test the *in vitro* spore reproduction of a commercial AM inoculum amendment, two sets of pots were established using the MycorrTree product. In accordance with manufactures suggested application, 3 ounces of inoculum were mixed with three gallons of a 2:1 sand/soil mix, which was then transferred to #5 standard pots (approx. 1,000 cm^3). The native grass big bluestem (*Andropogon gerardii*) was selected as a plant host. An initial set of 3 treatment and 3 control pots was established during fall of 1998. A second set of 7 treatment and 7 control pots was established 2 months later. Planted pots were maintained in an environmental growth chamber for approximately 6 months, before watering was stopped and plants allowed to senesce. Pots remained in the growth chamber for 1 month until dried, then soil from the pots was placed in plastic bags and stored at 4 °C.

Spore isolation

Spores were isolated from the pot cultured inoculum in a method similar that described in Tommerup and Kidby [18], however methods were altered to accommodate hydrophilic components found in MycorrTree TreeSaver (see results and discussion). Fifteen grams of soil were sieved to collect material between 250 and 38 μm in size. Sieved material (containing mycorrhizal spores) was further purified by sucrose density centrifugation. Spores were placed on grided filters and counted under a dissecting scope at approximately 60× magnification.

3.3.2 *Results and discussion*

Hydrophilic Mycorrtree TreeSaver components complicated isolation of spores cultured from this product. Once re-hydrated, larger chunks of Terrasorb gel and small pieces of yucca clogged sieves; hence isolation techniques were modified to account for hydrophilic components of the commercial inoculum product. Soil was re-hydrated for 1 hr in 500 ml of water and spore-containing material was filtered through two 500 μm sieves prior to spore isolation. In addition, wet sieving was lengthened to remove additional gel and plant material.

Spore data from the first and second sets were pooled for analysis, as results were similar. MycorrTree TreeSaver had significantly higher spore reproduction than the uninoculated controls, 25.6 spores per gram of dried soil compared to 2.4 spores per gram respectively (Figure

3.2). Spores were found in only one uninoculated control pot and likely resulted from culturing in a growth chamber used concurently for experiments by other researchers.

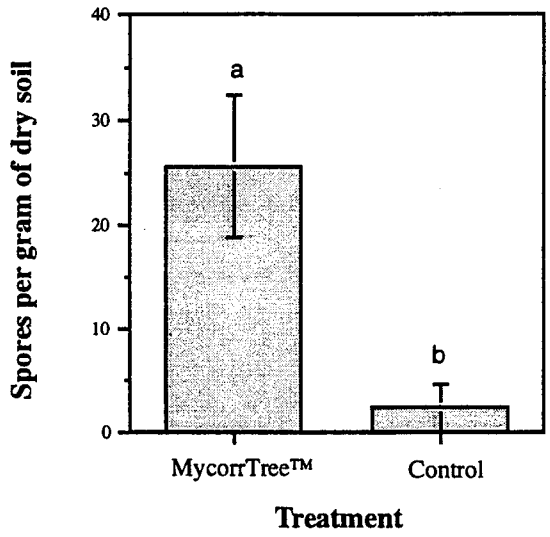

Figure 3.2. Spore number in inoculated pots. Bars are mean spore number per gram of dry soil ± 1 SE. Different letters represent significant differences between treatments ($p < 0.05$).

Multiple spore types were found in the MycorrTree inoculum product. Identification of these spores indicated that many were *Glomus etunicatum*. In addition, other *Glomus* species were found, but not identified to species.

3.3.3 *Conclusions*

We were able to successfully reproduce spores from MycorrTree TreeSaver product. Viable mycorrhizal spores were present in the parent commercial product; however, our isolation of 25 spores per gram cultured soil is based on a single small sample of MycorrTree transplant saver and can not be used to calculate an absolute value of the viable spores in the parent commercial inoculum.

The methods used to examine MycorrTree TreeSaver showed promise as a viability and MPN (most probable number) test of fungal propagules in commercial AM products. With more work on the culturing techniques of the potted host/symbiont system, a reliable method for assaying the inoculum potential of commercial AM inocula could likely be developed.

3.4 Recommendations

1) More specific culturing protocols for assaying commercial inoculum viability should be developed. Using a uniform testing procedure, more consistent data concerning AM inocula could be generated and a more accurate picture of several inoculum products could be analyzed.
2) By application of a uniform culturing protocol, a quality control testing system could be established for mycorrhizal products that would be used on a continuing basis to check viability of commercial products before being used at landscaping/planting sites.
3) Using a similar screening method as 2 above, soil of potential restoration sites should be tested for the presence of mycorrhizal propagules before application of costly commercial inoculum. Moreover, soil characteristics such as pH should be tested and compared to the requirements of mycorrhizal species present in commercial inoculum before application.
4) This research suggests that native soils may have potential as inocula in nursery settings with container grown stock and plantation grown tree seedlings. Further research on the inoculum potential of native soils and their ability to colonize landscaping stock would be of value to suppliers of MN/DoT landscape stock.

Chapter 4. Trunk Highway 280: the influence of soil amendments on landscaped plant establishment

4.1 Overview

The Minnesota Department of Transportation (Mn/DOT) was responsible for the construction of the Trunk Highway 280 corridor running from Interstate Highway 94 to Interstate Highway 35W in the city of St. Paul, Minnesota. In 1996, Mn/DOT completed a resurfacing and rebuilding project for TH280. As part of the project planning phase, several community input meetings were held. The plan to put up a sound barrier along the roadway was thought to be a good idea, however residents thought that it would look sterile. Mn/DOT therefore agreed that an emphasis should be placed on 'beautifying' the right-of-way land adjacent to TH280. Members of Mn/DOT's Division of Environmental Services developed a landscaping plan that included a stretch of TH280 approximately 2 miles long (Figure 4.1).

The horticultural landscaping was done with two factors in mind. First was the need to make the area aesthetically pleasing. To this end, the landscapers planned to integrate the planted vegetation with the richness of flora found in the surrounding neighborhood. Second, the plants would have to have high survivability in a relatively harsh environment with little or no maintenance. The soil present along TH280 has been greatly disturbed on a periodic basis, probably since the initial construction of the highway. Such disturbance has been shown to damage the soil's resident population of microorganisms [35, 36], which in turn can lead to lowered plant vigor [37]. Moreover, the soil is a non-homogenous patchwork of sand and gravel, with high pH, low nutrient availability, low levels of organic matter, and contamination from past industrial plants adjacent to the site. All of these factors are generally detrimental to plant establishment.

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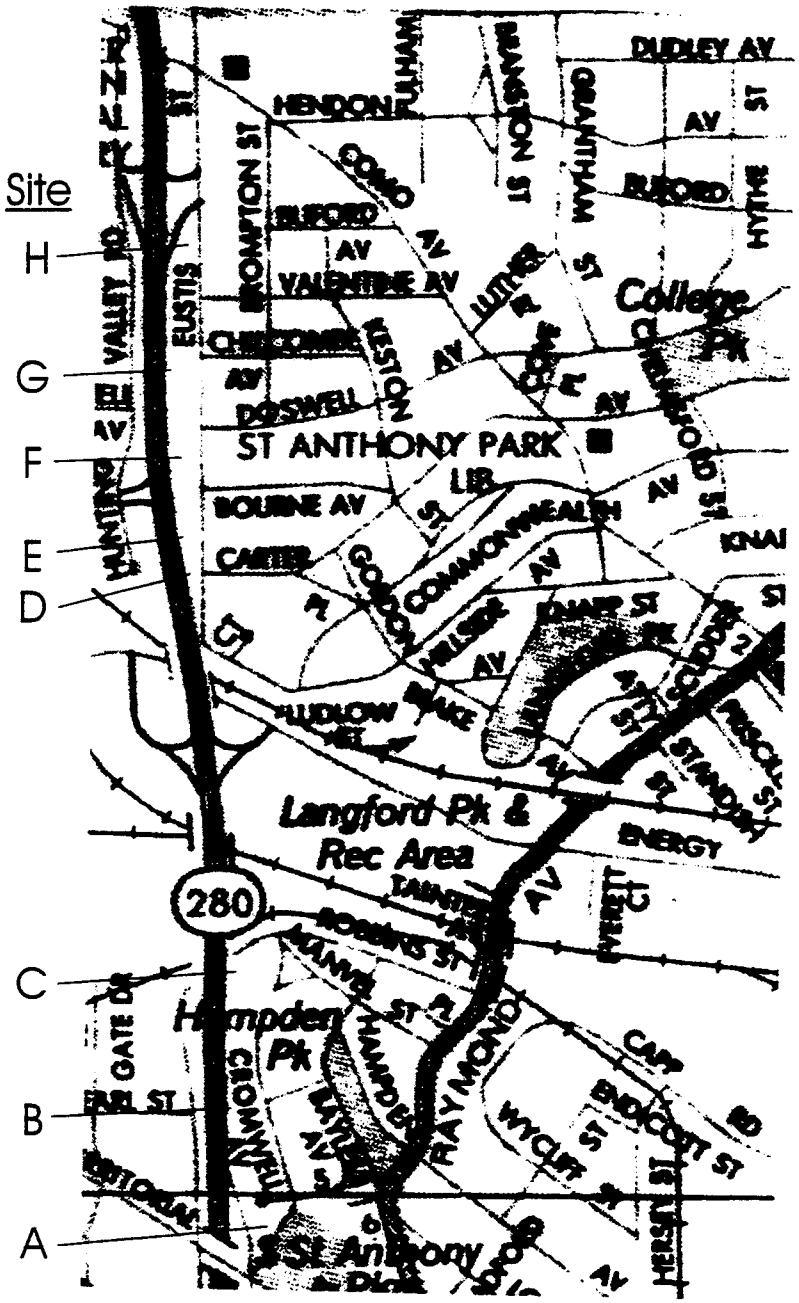

Figure 4.1. Trunk Highway 280, St. Paul, Minnesota. Landscaping was conducted at sites A-H.

Using these as their central criteria, the landscaper team devised a plan that included over 2000 perennial plants representing 32 species or varieties. To ameliorate the less than ideal soil conditions, a number of soil amendments were added at the same time as the nursery grown stock. Transplants received one of five amendment treatments, or remained unamended controls. The treatments were 1) Sulfur, 2) TreeSaver, 3) TreeSaver + sulfur, 4) Root Dip and 5) Root Dip + sulfur. The TreeSaver and Root Dip treatments represent two mechanisms of applying a commercially available mycorrhizal inoculum (MycorrTree™ TreeSaver™) from Plant Health Care, Inc. (Pittsburgh, PA). This inoculum contains propagules from ectomycorrhizal (EM) and arbuscular mycorrhizal (AM) species, as well as other substances that promote may plant growth and establishment. This inoculum can be added directly to the soil along with the transplant (the TreeSaver treatment), or can be made into a liquid slurry into which transplant roots are dipped (the Root Dip treatment). In areas with low densities of naturally occurring mycorrhizae in the soil, mycorrhizal inoculation can increase plant growth and health under low nutrient conditions [1]. Elemental sulfur reduces soil pH and can increase the availability of other soil nutrients to plants. Moreover, because many mycorrhizal species are also sensitive to pH [38], the sulfur amendment could also have indirect effects on plant growth mediated through the mycorrhizal amendments.

The purpose of this study was to determine whether the amendments improved plant establishment and growth. Two approaches were taken. First, overall survival and health were estimated across all planted species to see if there were broad treatment effects. Second, in depth analyses were performed for three species groups: the sumacs, the roses, and the oaks.

4.2 Materials and Methods

4.2.1 Landscaping timeline and personnel

Landscaping at the TH 280 site was designed by a Mn/DOT team led by landscape architect Carol Zoff Pelton. Project supervisors for the work were Tom Krier and Paul Juckel. The landscape contractor responsible for plant installation and soil amendments was Hoffman and McNamara, which was led on site by Gary Hoffman. Mn/DOT inspectors were Leroy Jacober and Chris Anderson.

Initial landscaping work was done in the fall of 1996. All planting beds were roto-tilled and beds receiving sulfur amendments had elemental sulfur applied and incorporated into the soil

and holes dug for larger balled and burlaped nursery stock. Plantings were completed in April and May of 1997. Mycorrhizal amendments were applied as either a dried powder in the planting hole for balled and burlaped stock or as hydrated root dip for bare root stock. All soil amendments and routine plant maintenance performed during by project were completed by the landscaping contractor.

The plant names used in this chapter were taken from the landscape plans provided to us by Mn/DOT. These plans were designated as state project number 6242-62 (TH 280). Plant names represent horticultural cultivars rather than scientific names given to the plants occurring in their natural habitats.

4.2.2 *Survival/health surveys*

Surveys of plant survival were conducted during 7/97, 9/97, 10/97, 4/98, 7/98, and 6/99. Each survey consisted of a walk though the entire site, counting the number of plants that were alive or dead, recording general health bed by bed, and noting any other issues that might affect the landscaping success on a long-term basis. Using all six census dates, percent survival was calculated for each species under each treatment. Plant health was estimated per bed on a qualitative 0-5 scale, where 0 = dead or nearly dead, 1 = unhealthy, high probability of death, 2 = unhealthy, questionable whether it will live, 3 = moderate condition, severe wear and tear, some disease, 4 = healthy, some normal wear and tear, 5 = perfect health.

To statistically analyze the final percent survival data, it was necessary to find pairs of treatments given to the same plant species that appropriately test for the effect of the amendment. Three treatment pairs were used to test for the effects of sulfur: Control/Sulfur, Root Dip/Root Dip + Sulfur, and TreeSaver/TreeSaver + Sulfur. To test for the effect of mycorrhizal amendment, 4 pairs of treatments were used: Control/Root Dip, Control/TreeSaver, Sulfur/Root Dip + Sulfur, and Sulfur/TreeSaver + Sulfur. No differentiation between the Root Dip and TreeSaver mycorrhizal treatments was made due to lack of replication. A species that was only given one treatment, or that had no appropriate treatment pairs was excluded from the analysis. The Wilcoxon signed ranks test at $\alpha = 0.05$ was used to test for significant differences. The 10/97 ranked health data was analyzed in the same manner.

4.2.3 *Sumac biomass*

Sumacs were chosen for a more intensive biomass study because after the first year it was not possible to perform survival analyses due to the rapid rhizomatous growth form, so a different measure of performance was needed. Additionally, when both smooth and staghorn sumac beds are considered, there is a useful balance of amendment treatments, allowing inference about the effects of amendments (Table 4.1).

Table 4.1. Sumac soil additive treatments.

bed	location	sumac species	soil additive treatment
1	Site A	smooth	root dip + sulfur
2	Site A	staghorn	sulfur
3	Site B	smooth	control
4	Site D	staghorn	sulfur
5	Site D-E	staghorn	control
6	Site E	staghorn	root dip + sulfur

Three $9m^2$ plots were selected at random from each sumac bed. The number of sumac stems within each plot was counted, and stems were measured for height, diameter, and number of branches. An estimate of stem biomass per plant was made using the following equation: $(\pi/4)*h*d^2 + [(b-1)/2]*(\pi/4)*h*d^2$, where h = main stem height, d = stem diameter, and b= number of branches. This equation approximates stem volume using the volume of a cylinder, and assumes that the average branch length is one half the length of the main stem. When the there are no branches but the main stem, b= 1, and the term $[(b-1)/2]*(\pi/4)*h*d^2$ becomes zero. Stem volume per plant was then summed for each plot, yielding a biomass estimate in the units of cm^3 sumac stem/m^2. Sumac biomass was compared statistically among treatments using ANOVA at $\alpha = 0.05$.

4.2.4 *Mycorrhizal colonization of rose and oak*

Soil and root samples were taken from each of the six rose beds on an annual basis. The 1997 samples were sent to the University of Minnesota Research and Analytical laboratories, and measured for nitrate, ammonium, phosphorus, moisture, organic material, and total organic carbon. The 1999 samples were used for mycorrhizal testing, as these samples had the largest volume of rose roots. After isolation from the soil, half the root material from each sample was stained for quantification of AM mycorrhizal colonization, and half the roots remained unstained

and were examined under a dissecting scope for ectomycorrhizal colonization. The roots to be stained were cleared in 10% KOH overnight, then bleached in H_2O_2 for six hours before acidification and staining in 0.05% w/v trypan blue [39, 40]. The stained roots were then mounted on microscope slides and examined for colonization at 100-400X magnification using magnified intercept method [21]. The unstained roots were examined qualitatively under a dissecting microscope (50× magnification) for ectomycorrhizal colonization.

Oak roots were sampled at six sites on an annual basis. The 1997 samples were sent to the University of Minnesota soil testing laboratory, and measured for nitrate, ammonium, phosphorus, moisture, organic material, and total organic carbon. Oak roots were isolated from the 1998 and 1999 samples, and stored in 50% ethanol prior to examination. Oak roots were assayed for the presence of ectomycorrhizae under a dissecting microscope. When ectomycorrhizae were found, samples were placed on a slide and viewed under a compound microscope to determine mantle characteristics and associated fungal hyphal characteristics. Due to the small volume of oak roots in most samples, ectomycorrhizal colonization levels were not quantified.

4.3 Results and Discussion

4.3.1 *Survival/health surveys*

Survival was at or near 100% for almost all species under all treatments (Table 4.2), indicating that plant selection by the landscape design team and plant installation and maintenance by the landscape contractor were ideal. There were a few exceptions, however. On two occasions, landscaped plants were accidentally damaged by Mn/DOT personnel spraying Tordon™ (picloram) and 2,4,D herbicides for control of perennial sow thistle, a noxious weed which must be controlled under state weed laws. Twenty-two Hansa roses and approximately 40 Boston ivy plants were killed by the herbicide, and later replanted by the landscaping contractor. The affected beds were excluded from the statistical analysis, because the cause of mortality was not related to soil amendment treatment. With these exclusions, only three species with appropriate treatment pairs showed any differential mortality (Table 4.3). Consequently, no statistical differences were found in plant mortality as a result of soil amendment.

Table 4.2. Percent survival of plantings along the T.H. 280 corridor, sorted by plant species.

Species	Control		Sulfur		Root Dip		Root Dip + Sulfur		TreeSaver		TreeSaver + Sulfur	
	#[a]	%[b]	#	%	#	%	#	%	#	%	#	%
Arborvitae, Technae	5	100							5	100		
Buffaloberry, Silver	40	100	105	100							50	100
Cedar, Eastern Red	5	100	10	100					5	100	5	100
Coffee Tree, Kentucky											10	100
Dogwood, Cardinal Red	50	98			35	100						
Dogwood, Grey					80	100						
Ivy, Boston[c]			45	22.2	20	95	25	96				
Ivy, Englman	10	100	10	100			10	100			10	100
Juniper, Fairview											2	100
Juniper, Seagreen									90	100		
Maple, Emerald Queen Norway	1	100									1	100
Maple, Norway	1	100									3	100
Maple, Oregon Pride	2	100									3	100
Oak, Bur	5	100	6	100					5	100	7	100
Olive, Russian			80	100			95	90.5				
Peashrub, Siberian[d]												
Pine, Austrian	16	100	6	100					9	100	4	100
Pine, Ponderosa	3	100									3	100
Pine, Scotch	11	100	3	100					5	100	11	100
Plum, American	15	100					50	98				
Poplar, Siouxland					5	80						
Potentilla, Abbotswood					35	100						
Rose, Alba Plena	35	100							35	100	30	100
Rose, Hansa			75	100							75	70.7
Rose, Thersa Bugnet											35	100
Snowberry, White	55	100										
Spirea, Ash Leaf							30	100				
Sumac, Fragrant[a]												
Sumac, Smooth	20	95					40	92.5				
Sumac, Staghorn	36	94.4	152	81.6			32	96.9				
Willow, Dwarf Arctic Blue					65	98						
Willow, Flame							10	100				

[a] Total number of plants surveyed

[b] Percent survival

[c] The majority of losses in this species were due to herbicide spraying.

[d] This species was not surveyed.

Table 4.3. Comparison of survival rate with or without soil amendment.

Amendment	Species with higher survival when amended	Species with lower survival when amended	Species with equivalent survival when amended	P value
Sulfur	2	1	9	---[a]
Mycorrhizae	0	0	5	---

[a] Non-zero sample sizes were not large enough to calculate p values.

Differences in health were also relatively slight among treatments. However, 7 out of 11 species were ranked somewhat higher in health when given sulfur, corresponding to a p-value of 0.09 (Table 4.4). This gives some evidence that sulfur may have improved plant health, but not terribly strong evidence. Only 4 out of 11 species showed greater health in the mycorrhizal amended beds, giving no evidence that mycorrhizal amendments improved plant health in this experiment.

Table 4.4. Comparison of health rankings with or without soil amendment.

Amendment	Species with higher health rankings when amended	Species with lower health rankings when amended	Species with equivalent health when amended	P value
Sulfur	7	2	2	0.09
Mycorrhizae	4	3	4	0.50

Overall, the plant survival and health parameters used in this survey were rather coarse measures for detecting differences among treatments: treatment effects would not only have to be very strong to show up, but also be consistent across a large number of plant species, and a variety of environmental conditions. Moreover, the use of plant species as the experimental unit in this analysis limited the sample size rather severely, and further restricted the power of detecting effects. However, given the lack of independence of plants growing in a common bed, it seemed inappropriate to use individual plants as experimental units.

The initial mortality observed in sumacs was more than compensated for by extensive clonal growth. In fact, it became impossible to calculate sumac survival in the later surveys because of the tremendous number of clonal offspring. Many of the rose varieties have also sent forth numerous ramets, in many cases completely filling in the beds in which they are planted. However, this extremely vigorous growth may be negatively affecting other planted species. In a number of instances, sumac has nearly overtopped nearby planted tree species, making it unclear whether the trees will get enough light to be effective competitors. Moreover, sumac recruits have been found across the barrier wall, indicating either clonal reproduction under the wall, or bird/mammal seed dispersal. The northernmost plantings of Alba plena roses (Site H) are invading nearby seagreen juniper and abbotswood potentilla beds.

Additionally, weedy forb and tree species have naturally recruited into a number of beds, competing with the planted species. Chinese elm and extremely vigorous crown vetch have

invaded the southernmost Hansa rose beds (Site A), wild grape has overgrowing some Alba Plena roses near the barrier wall, and box elder recruits were commonly found in the dogwood plantings. It should also be noted that the native prairie seeding between landscaping beds in Sites A-B was generally unsuccessful. While there are scattered native species present (side-oats grama, common ox eye, purple prairie clover), the interspersed vegetation is dominated by weedy mustards and mint species, along with crown vetch. The overall appearance in this, and many other areas of the corridor, is strikingly weedy. It may be worthwhile to re-seed the prairie species in these areas, both to improve their appearance and to protect the landscaped beds by decreasing the nearby sources of weedy invasive species. Furthermore, a maintenance regime or herbicide and mowing treatments would be required to allow establishment and long term survival of newly seeded areas.

4.3.2 *Sumac biomass*

Statistical analysis showed that biomass was greater for staghorn sumac than for smooth sumac ($p = 0.0017$, Figure. 4.2). This made it inappropriate to lump both species of sumac together when comparing soil amendment treatments. Because the sulfur treatment was only applied to staghorn sumac, whereas the other two treatments were applied to both staghorn and smooth sumac beds, the smaller biomass of smooth sumac can make it appear that the other two treatments were less effective than the sulfur treatment. Consequently we limited our assessment of soil additive effects to staghorn sumac plots, only.

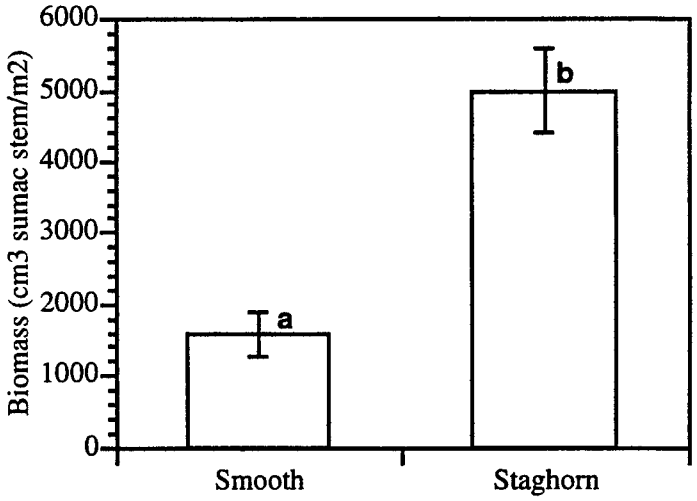

Figure 4.2. Mean (± 1 SE) biomass of smooth vs. staghorn sumac per m^2 plot, fall 1998.

We did not find differences among the treatments for staghorn sumac (p=0.181, Figure. 4.3). It should be noted that statistically this experiment had very low power, meaning that true differences between treatments would be very hard to detect due to the small number (4) of staghorn sumac beds.

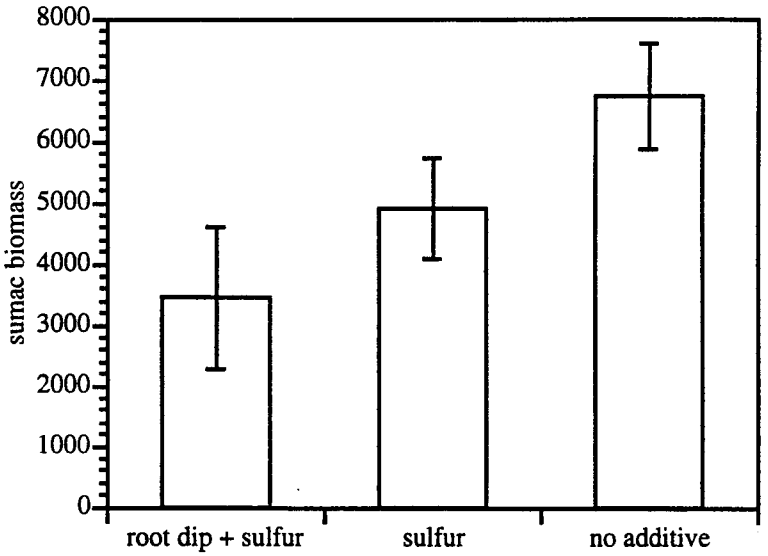

Figure. 4.3. Mean (± 1 SE) biomass of staghorn sumac per m^2 in beds given different soil additives, measured fall 1998.

4.3.3 *Mycorrhizal status of rose and oak*

Colonization of rose roots by AM fungi was widely variable from planting to planting, (Figure 4.4). This variation does not correlate well to the applied soil amendments. Bed H2, which received the TreeSaver treatment, had the highest level of mycorrhizal colonization, but the next highest colonization was found in bed H1, which was not inoculated. Colonization was much lower (approximately 20%) in beds A1, B1 and D1, which all received sulfur and/or TreeSaver, and almost completely absent from bed C1, which also received sulfur and TreeSaver. The fact that beds H1 and H2 had similarly high colonization implies that site conditions may be more important than amendments in determining the colonization exhibited by rose. This hypothesis is corroborated by the soil nutrient data collected from each bed (Table 4.5). Site C had the highest levels of nitrate and phosphorus, whereas site H had the lowest levels of these nutrients. High nutrient availability has repeatedly been shown to inhibit mycorrhizal

colonization [41, 42]. Alternatively, these sites probably also differed markedly in their pre-construction vegetation composition, so the naturally occurring inoculum in the soil may have been different from site to site as well.

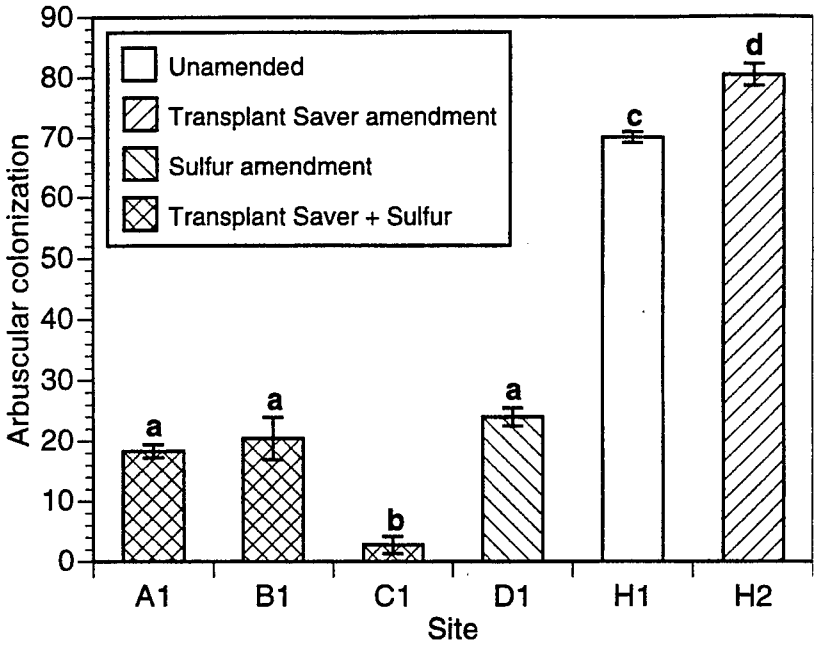

Figure 4.4. Mean (± 1 SE) arbuscular colonization of roses planted along TH280 in October 1999. Each column represents a different bed; columns that share are letter are not significantly different at $\alpha = 0.05$.

Table 4.5. Soil measurements for rose plantings along TH280, July 1997.

Site	A1	B1	C1	D1	H1	H2	Mean
NO_3 (ppm)	34.7	26.5	42.8	26.8	24.0	17.2	27.7
NH_4 (ppm)	7.0	14.5	5.1	11.4	2.1	1.4	6.9
Olsen-P (ppm)	123	122	250	122	86	100	133.8
% moisture	23.6	23.7	26.0	28.0	21.2	20.4	23.8
% OM	6.25	6.92	7.48	8.69	5.80	5.26	6.73
% Carbon	3.42	4.08	4.12	4.69	3.87	3.26	3.91

Based on this data, it is impossible to say exactly what combination of factors most influenced colonization levels, but it is clear that underlying site to site variation makes it difficult to draw conclusions about the efficacy of the amendments. To truly test the effects of

amendments, both amended and unamended treatments need to be implemented at matched sites, in order to control for environmental variation. Site H is a good example. Beds H1 and H2 are quite similar in their soil nutrient content, indicating that the higher level of colonization in bed H2 than bed H1 could potentially be attributed to the addition of the TreeSaver treatment. However, it would be extremely premature to make this conclusion based on a single replication. In future tests of soil amendments, an effort should be made to have both amended and unamended treatments at each site. The experiment would then have the power to detect amendment effects despite underlying environmental variation.

Ectomycorrhizae were found in oak roots from all sampled sites (Table 4.6). The color of the mycorrhizae could not be used as an identifying feature because color is lost during storage in ethanol. However, the mantle type could be discerned as well as associated hyphae. For all five 1998 samples examined, the ectomycorrhizal morphotype corresponded to morphotype B, described in the pot culture study of oak ectomycorrhizae (chapter 3). This morphotype was common in oaks grown in unsterilized field soil, and was presumably extant at the site prior to landscaping. Morphotype B was present in samples from 5 out of 6 sites in 1999. The sixth site had morphotype A ectomycorrhizae, as described in chapter 3, as did three of the sites that also had morphotype B. Under pot culture, morphotype A was found at very low levels in oaks grown in sterile soil, either with or without commercial inoculum addition. This suggested a greenhouse contaminant, rather than colonization via the commercial inoculum or native soil. The presence of morphotype A in field sites that did not receive mycorrhizal amendment (sites A4 and B) strengthens the conclusion that it was not introduced with the commercial inoculum product. There is little evidence that mycorrhizal inoculation substantially affected oak colonization in the field.

Table 4.6. Ectomycorrhizal morphotypes found on oak roots from TH280.

Site	Treatment	Ectomycorrhizal morphotypes	
		1998	1999
A1	TreeSaver + Sulfur	morphotype B	morphotype B, A
A2	TreeSaver + Sulfur	morphotype B	morphotype B
A3	Sulfur	morphotype B	morphotype B
A4	Sulfur	morphotype B	morphotype A
B	Unamended control	morphotype B	morphotype B, A
H	TreeSaver	morphotype B	morphotype B, A

4.4 Conclusions and Recommendations

1) Regardless of soil amendment treatment, plant growth was vigorous and showed high survival in the landscape plantings along TH280, indicating that plant selection by the landscape design team and plant installation and maintenance by the landscape contractor were superb.
2) It may be worthwhile to re-seed the prairie species between the landscaped beds, both to improve their appearance and to protect them by decreasing the nearby sources of weedy invasive species.
3) To improve the sensitivity of future tests of soil amendments, a "blocked" design should be undertaken, where each landscaping bed in the test is split into amended and unamended portions. Ideally, at least three such beds should be used per plant species tested.
4) Soil phosphorus and nitrogen should be measured at each site before addition of mycorrhizal amendments. If soil nutrient content is high, addition of mycorrhizal inoculum is unlikely to be of value.

Chapter 5. Use of soil amendments in a prairie restoration

5.1 Overview

The harsh environment of many prairie restoration sites and resulting poor plant growth have led vegetation managers to examine new techniques and amendments for restorations at such sites [43, 44]. One suggested amendment is application of arbuscular mycorrhizal (AM) fungal inoculum. AM fungi are root-borne fungi that aid plants in nutrient uptake [1]. Plants often form symbiotic associations with AM fungi by providing them with carbohydrates produced during photosynthesis. Unfortunately, prairie restorations are often conducted on sites that have been severely disturbed and lack a viable population of mycorrhizal fungi [2, 3]. The lack of fungal propagules and subsequent lack of mycorrhizal symbiosis are hypothesized to result in reduced fecundity of native species at restoration sites.

Several studies have shown that the majority of native prairie plants form facultative or dependent symbiosis with arbuscular mycorrhizal fungi [45, 46]. The large number of facultative and obligate mycotrophic prairie plants suggests that mycorrhizal associations confer added fitness to host plants and are therefore a competitive advantage for mycorrhizal plants. Many prairie plants have a very coarse root system (many large central roots with few smaller branch roots), which is less efficient at obtaining nutrients. Therefore, these plants would likely benefit from fungal associations and the finely branched hyphae of AM fungi.

Many non-native, 'weedy' plants have the ability to extract nutrients from soil without mycorrhizal associations [47]. Their fibrous root system, with many fine roots, is thought to function in the same role as mycorrhizal fungi. Two major plant families, Brassicaceae and Chenopodaceae, whose members are primarily non-mycorrhizal, contain some of worst 'weeds' in plant communities [1, 48].

It therefore appears that the balance between native prairie species and non-native 'weedy' vegetation could hinge greatly on the presence or absence of mycorrhizal fungi. The application of additional AM fungal propagules could shift balance of the restoration community towards a more diverse native plant community.

Initial tests of mycorrhizal amendment applications during prairie restorations have demonstrated positive results [4, 5]. Noyd et al. [4] found that total cover was increased during the second year following restoration establishment. Smith et al. [5] noted a significant increase

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in the native grass cover during the second year. These findings suggest that AM inoculation altered the vegetation present and possibly the competitive balance of plants during succession at restoration sties. We hypothesized that addition of mycorrhizal inoculum would promote a more diverse native plant community.

Fertilization has been suggested as an alternative method of supplying native plants with nutrients, which may be less costly than mycorrhizal inoculum. Little data exists on fertilizer application at restoration prairies, however studies have examined nutrient application at early successional prairies [49]. 'Weedy' annual plants tend to be resource (nutrient) dependent, growing and reproducing during periods of high resource (nutrient) availability. Whereas native plants apply many of their nutrient and photosynthate reserves towards long-term survival adaptations, such as a deep taproot system or extensive fibrous root system. We hypothesized that direct application of nutrients would especially benefit plants that more rapidly utilize nutrients (fast-growing weedy annuals), thereby shifting the competitive balance towards weedy annual species.

Our group established an experimental prairie restoration at a wet prairie site in central Minnesota to further examine methods of increasing nutrient availability to native plants during restoration. We were interested in the effect of mycorrhizal and fertilizer amendments on the plant community. Several mycorrhizal and fertilizer amendments were combined with two application techniques during establishment of the restored prairie. Mycorrhizal inoculum amendments were selected based on their availability to researchers and restorationists. Fertilizer treatments were chosen to represent methods and rates of fertilization that are currently used by vegetation managers [8].

Four mycorrhizal treatments were selected for installation at our site. Inoculum produced by our lab [17] was applied in two treatments, a broadcast application and a row-planted application. Our inoculum contained regional ecotypes of mycorrhizal fungi, which we thought might form better associations with native plants than other non-local AM fungal amendments. A non-local commercially produced inoculum containing AM fungal propagules and plant growth promoting substances was also examined [50].

Fertilizer treatments included a standard inorganic mineral fertilizer and a sulfur-coated urea slow-release fertilizer, which were compared to control plots receiving no fertilization. Inorganic mineral fertilizers have been commonly used in agriculture for many years. Initial use

of mineral fertilizers on roadside plantings likely originated with non-native plantings, which benefited from nutrient amendment. However, the use of fertilizers has been continued as native plantings have begun to be used along roadsides. Slow release fertilizer is a relatively new fertilization method that is significantly more expensive than tradition mineral fertilizers. It provides a timed release of nutrients over several weeks or months and offers a more balanced application of nutrients, mimicking natural nutrient release by soil decomposition processes. We were interested in the ability of slow release fertilizer to provide a low dosage of nutrients targeted to native plants, in contrast to mineral fertilizers, which release high concentrations of nutrients for brief periods of time. We hypothesized that slow release fertilizer would function to promote growth of planted native species that utilize nutrients at a slower constant rate.

In addition to the effects of fertilizer on the plant community, we were also interested in the effects of fertilization on AM fungal community. Studies have demonstrated that high levels of fertilization have detrimental effects on mycorrhizal spore numbers and colonization [51]. Many of these studies examined agricultural host plants species or AM fungal species at sites receiving yearly doses of fertilizer [24, 26]. However, few studies have examined the effects of fertilization on AM fungi during establishment of a native prairie. We were interested in examining how a one-time fertilization would affect the mycorrhizal community at a newly restored prairie.

During our restoration, a modification in the seed mix was made to observe whether a higher seeding rate of forbs would result in a restoration more closely resembling remnant prairie sites. The seed mix modification was suggested by Dwayne Stenlund, the Mn/DOT technical liaison for our project. The percentage of forbs in the specified seed mix under-represented the amount of forbs found in remnant prairies and could explain the low level of forbs found in some prairie restoration communities. Therefore, a seed mix with a higher percentage of forb seed was applied to the site for a rough comparison to previous restorations completed with a lower number of forb seeds per hectare.

The plant and mycorrhizal communities were examined by analysis of several factors. Vegetation parameters included species number per plot, aboveground biomass of vegetation, number of individual of each species and average height of each species. Mycorrhizal data analyzed consisted of AM root colonization of root tissue isolated in treatment plots. Environmental data such as temperature, water table levels, and rainfall were also considered.

Using this data, we examined how mycorrhizal and fertilizer amendments altered the plant community structure. We specifically looked at species occurrence, plot diversity and overall biomass to determine whether the soil amendments aided the native component of a prairie restoration.

5.2 Materials and Methods

5.2.1 *Site selection and design*

A restoration site was selected on Mn/DOT owned land near Shakopee MN (44°46' N 93°24' W) (Figure 5.1). Prior to construction of Minnesota State highway 101, a mobile home park covered the site. Following completion of the highway, Mn/DOT contracted a prairie and wetland restoration on the 13 acre Mn/DOT owned right of way, using native prairie and wetland seeds and plants. By 1997, the originally restored prairie area had few remaining native plant species. Vegetation was sparce with patchy monocultures of native species surrounded by large areas of mixed 'weedy' vegetation.

A large fenced enclosure, uniform soil, and relatively even vegetation made the Shakopee location a prime site for restoration. The 13-acre site was constructed to function as a water-storage pond with attached prairie floodplain. During our experiment the prairie floodplain had some standing water for a few days during each year.

Treatments were applied in a random block design with 5 replicates (Figure 5.2). Five blocks were laid west to east along the southern side of the site to allow as much space as possible between the experimental plot and the water basin on the north side of the site. Each treatment plot was 2 m by 2m with a buffer zone of 1-m surrounding each plot (2 m between treatment plots). A further buffer zone of approximately 4-m was planted with native grasses around the entire research site.

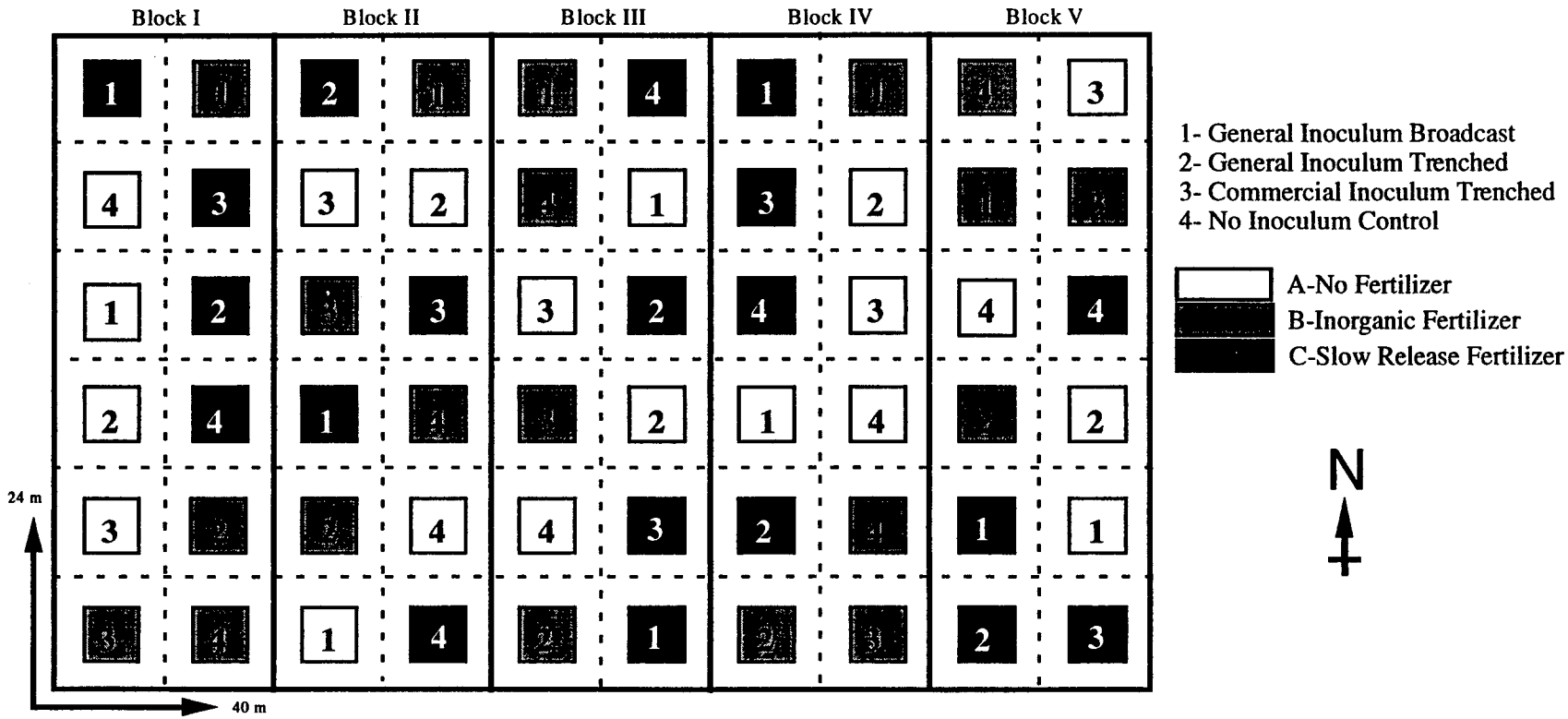

Figure 5.2 Treatment plot layout at the Mn/DOT Shakopee research site. Plots were laid out in 5 blocks west to east. Each plot had one of three fertilization treatments (no fertilizer, inorganic fertilizer or slow-release fertilizer) and one of four mycorrhizal treatments (lab produced inoculum broadcast, lab produced inoculum trenched, commercial inoculum trenched, and no inoculum control).

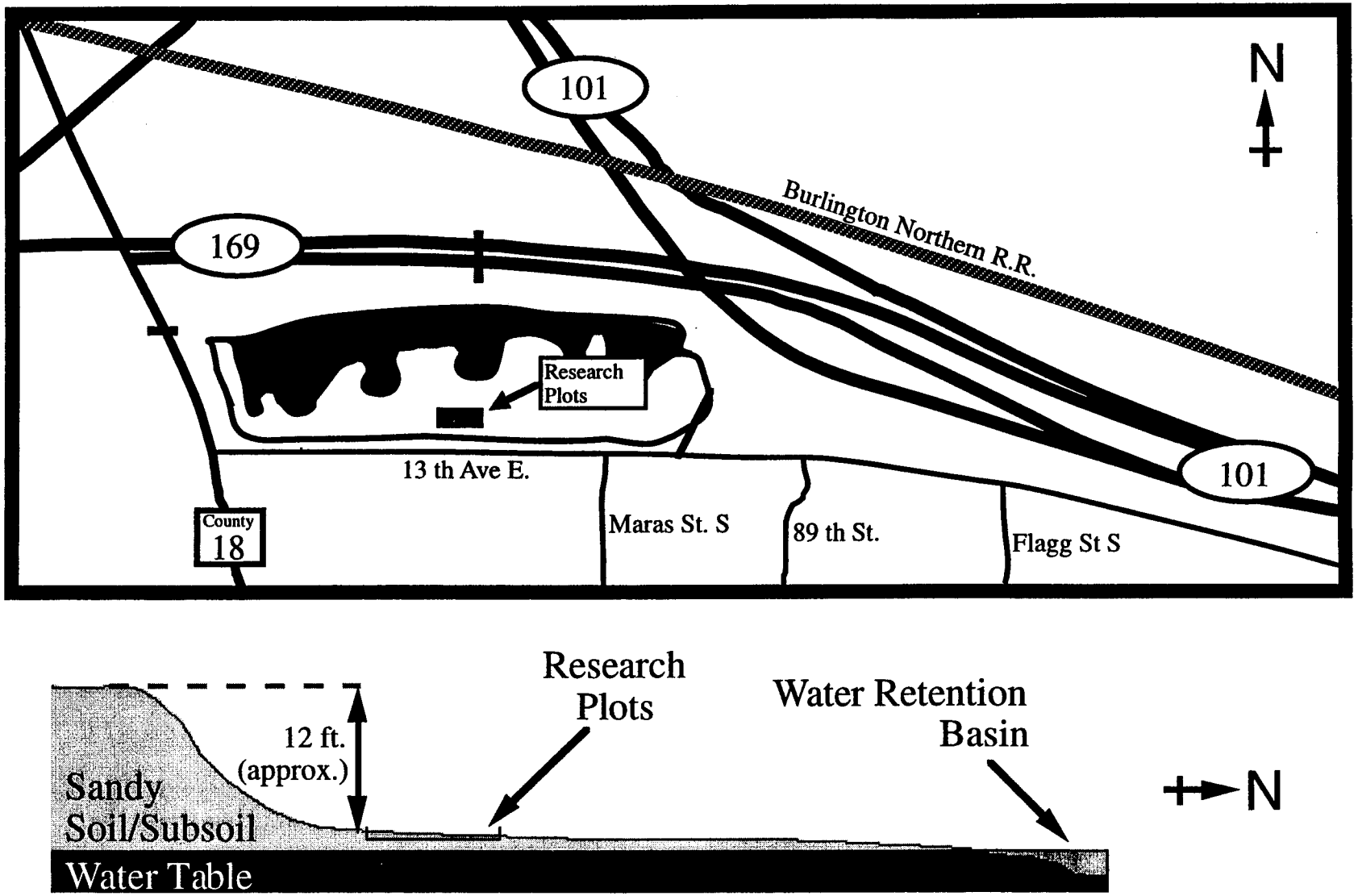

Figure 5.1 Shakopee research site. Top panel- Location of the Shakopee research site three miles SE of Shakopee, Mn at the highway 101/169 interchange. Bottom panel- Cross section showing the location of the plots at the roadside site.

5.2.2 *Site preparation*

The site was tilled over a two-day period with a commercial walk behind roto-tiller. All areas of the plot were tilled twice at intersecting angles. Light rain after the first night, moistened the dry sandy soil and provided an excellent seeding bed. Application of glyphosate herbicide was not deemed necessary, as pre-existing vegetation was sparse.

5.2.3 *Native seed mixture*

Seeds were selected using the 1996-97 Mn/DOT seeding guidelines [8], with mix 15A selected as the basis for our seed mix. A regional guide to species [52] was also consulted to verify the presettlement range of each species. The forb component of the mix was increased from 2% to 10% (see above).

Twenty forbs and seven grass species were planted (Table 5.1). Native seeds were purchased from Prairie Restorations Inc. (Princeton, MN), Prairie Moon Nursery (Winona, MN), and/or Peterson Seed Company (Shakopee, MN) with emphasis on obtaining local genotypes of each species. Regreen™ a sterile hybrid of winter wheat and western wheat grass was included in the seed mix as a cover crop. Seeds for each plot were pre-measured and packaged in the lab prior to planting. A simple test of germination was conducted in flats containing a mixture of perlite and vermiculite. Most grass and several forb seeds germinated within 21 days of seeding.

Table 5.1. Planted seed mixture and abundance of planted species.

Common name	Scientific Name	Planting Rate kg/ha	Species Abundance[a]	Notes[b]
Grasses				
Big bluestem	*Andropogon gerardii*	4.5	***	
Canada wild-rye	*Elymus canadensis*	3.5	-	1
Indian grass	*Sorghastrum nutans*	3.5	***	
Little bluestem	*Schizachyrium scoparium*	2.5	***	
Re-Green (replaces oats)		18	**	
Sideoats grama	*Bouteloua curtipendula*	3.5	*	
Slender wheat-grass	*Agropyron trachycaulum*	1	-	1
Switch grass	*Panicum virgatum*	1	-	2
Forbs				
Black-eyed susan	*Rudbeckia hirta*	0.25	***	
Blue vervain	*Verbena hastata*	0.25	**	
Butterfly milkweed	*Asclepias tuberosa*	0.25	-	
Canada milkvetch,	*Astragalus canadensis*	0.25	***	
Common ox-eye	*Heliopsis helianthoides*	0.25	-	3
Grey-headed coneflower	*Ratibita pinnata*	0.25	**	
Hoary vervain	*Verbena stricta*	0.25	**	
New England aster	*Aster novae-angliae*	0.25	**	
Ohio spiderwort	*Tradescantia ohiensis*	0.25	-	3
Partridge pea	*Chamaecrista fasciculata*	0.25	**	
Purple prairie clover	*Dalea purpureum*	0.25	*	
Roundheaded bushclover	*Lespedeza capitata*	0.25	**	
Showy goldenrod	*Solidago speciosa*	0.25	*	
Showy penstemon	*Penstemon grandiflorus*	0.25	-	
Showy tick-trefoil	*Desmodium canadense*	0.25	**	
Smooth-blue aster	*Aster lavies*	0.25	**	
Stiff goldenrod	*Solidago rigida*	0.25	**	
Tall blazingstar	*Liatris pycnostachya*	0.25	-	
White prairie clover	*Dalea candidum*	0.25	-	
Wild bergamot	*Monarda fistulosa*	0.25	***	

a- Frequency ratings

*** abundant

** common

* uncommon

- not found

b- Notes

1- Small numbers of immature grasses may have been missed

2- Seed tested after planting and found not viable

3- Not seen in plots, however found immediately outside them

5.2.4 *Mycorrhizal and fertilizer treatments*

Three fertilization treatments and four mycorrhizal treatments were used in all combinations for a total of 12 different combinations of fertilizer and mycorrhizal inocula.

Mycorrhizal treatments were prepared and packaged in the lab prior to installation at the field site. Commercially produced inoculum, Flowerbed Inoculant™ (Plant Health Care, Inc. Pittsburgh, PA) was applied in seed rows at a level of 0.147kg/m^2 [1470 kg/ha]). FlowerBed Inoculant™ obtained from Plant Health Care, inc. was packaged into 585g aliquots for each plot. Flowerbed inoculant contains at least 4 AM species and at the manufacturer's suggested application rate yields approximately 4300 spores/m^2.

Arbuscular mycorrhizal inoculum produced by our lab was a mixture of 3 batches of inoculum produced between 1995-1997, from soil initially taken from the Crosstown remnant prairie site located at the intersection of Trunk Highway 62 and Trunk Highway 55, approximately 13 miles from the Shakopee restoration site. Local AM inoculum produced by our lab was tested in two application methods; one with both seed and inoculum applied in rows much like a native seed drill (0.63 kg inoculum per m^2 [6300 kg/ha]), and another with both seed and inoculum evenly broadcast over the treatment plots (0.63 kg inoculum per m^2).

Sterilized lab produced inoculum (0.63 kg/m^2 dry weight) was used as control for addition of inoculum. In order to supply a component of the microbial community to the control inoculum, 6 L of microbial rinse was added. The microbial rinse was produced by straining 6 L of water through 5 kg of unsterilized inoculum soil, followed by filtering the rinse over a 25 µm sieve to prevent mycorrhizal spores from being reintroduced into the control inoculum.

A 6-24-24 (N-P-K) inorganic mineral fertilizer was applied at 225kg/ha as per Mn/DOT guidelines (Howe Fertilizer, Minneapolis). An encapsulated slow-release 22-5-10 fertilizer with a three-month release time was also used, with an application rate of 305 kg/ha according to manufacture's suggestion (Howe Fertilizer, Minneapolis). The encapsulated fertilizer also contained micronutrients. An unfertilized control treatment was established for comparison to the two fertilization treatments.

5.2.5 *AM inocula and fertilizer application*

Fertilization and planting of the research plots occurred from June 26th to July 2nd, 1997. Soil was raked prior to application of treatments. All fertilizer treatments were applied

immediately before seeding. Fertilizers were broadcast spread over the 4 m^2 plots, then raked into the top 4 cm of soil.

Broadcast planting involved spreading native seeds and AM inocula over the 4 m^2 treatment area. Seeds and inoculum were raked into the soil to an estimated average depth of 1 cm. Use of a seed drill was simulated by planting 11 rows of seeds per plot. Rows were hand/trowel dug to a depth of 3 cm. Inoculum and seed were dispensed evenly into the 2-m long rows and covered to a depth of 1 cm with soil. Planting of the buffer zones between and surrounding the plots was done by the broadcast method.

Planting and inoculation of the treatment plots was completed on June 26 and 27th. Inter-plot and surrounding buffer zones were seeded on June 30 and July 2nd respectively.

5.2.6 *Soil and root sampling*

Soil samples were collected to a depth of 15 cm with a 2.5-cm diameter soil corer. Samples were placed on ice after collection and then stored at 4° C until processing. Yearly samples taken each growing season during the 3rd week of September. A total of 10 soil cores per plot were collected, homogenized, and divided to provide samples for nutrient analysis, spore isolation, and root colonization. A sample of soil from the 5 replicates of each treatment combination was composited and used in nutrient analysis. Composited soil samples were frozen at -20° C until analyzed by the University of Minnesota Research and Analytical Laboratories. Samples collected for spore isolation were dried down and stored at 4° C for future analysis. An additional set of soil samples was also taken on selected plots shortly after planting to assess possible movement of nutrients due to heavy rainfall shortly after fertilization. These samples were analyzed only for N, P, and K concentrations using a less precise 'farmer's test'.

5.2.7 *Root staining and colonization analysis*

Roots were isolated from the 1998 and 1999 samples using a 2 mm sieve, washed free of debris, and preserved in 50% ethanol. Isolated roots were cleared for 24 h in 10% KOH, acidified for 1 h in 1% HCl, placed in 0.05% w/v trypan blue for 24 h, and destained in acidic glycerol [39, 40, 53]. Subsamples were then randomly selected for determination of percentage colonization using the magnified intersections method [21] at 100-400× magnification.

Approximately 200 intersects per plot per sampling period were evaluated for the presence of AM fungal structures, including arbuscules, vesicles, and hyphal coils. Total percentage colonization for each year was analyzed statistically using blocked, 2 way ANOVA at $\alpha = 0.05$, followed by Fisher's PLSD multiple comparisons test [SYSTAT v 9.0].

5.2.8 *Vegetation sampling*

Plant material was collected during the 3rd week of September each fall. A 10-cm by 100-cm strip of vegetation was harvested from each plot each year. All aboveground material was taken down to bare soil. Plant material from individual treatment plots was frozen within 10 hours of harvesting for preservation. Individual plant samples were thawed in water, counted, and measured over a 4-month period. Following counting, plants from each plot were sorted by species, grouped into related types (see below), packaged in paper bags and placed into a 65° C drying oven. Dry weight measurements were taken after a minimum of 3 weeks drying.

During sorting, plants were separated into one of 5 categories; desired grasses, desired forbs, undesired grasses, undesired forbs, and litter. Unidentified plants were sorted based on morphology and similarity to known species. Specimens of unknown species were pressed for later identification.

ANOVA of vegetative data was performed using Systat software (Systat Inc., Evanston, IL). Descriptive statistics and graphs were produced with Statview 4.5 (Abacus Concepts, Berkeley, CA.). Diversity was calculated using the Shannon-Weaver Index (H') [54]:

$$H' = - \Sigma p_i \log p_i$$

Where p_i is the proportion of species I in the vegetation sample

Species that were very uncommon and difficult to properly identify were not used in diversity calculations, 2.7% of the total number of plants were not included in diversity calculations. Excluded species were those found in less than 10% of treated plots.

5.3 Results

5.3.1 *Installation and sampling of the restoration site*

Installation of the research plots was completed in two days and buffer zones were planted four days later. Shortly after installation, a series of severe thunderstorms occurred throughout the region, with reports of 2 inches of rain per hour. Soil samples were taken to determine whether fertilizer treatments remained in the areas to which they were applied. Tests of N, P, and K levels indicated that areas treated with fertilization had more nutrients than non-treated plots (Figure 5.3). Increased growth in slow release fertilizer plots also suggested that fertilizer treatments had not been uncovered and moved during the storms.

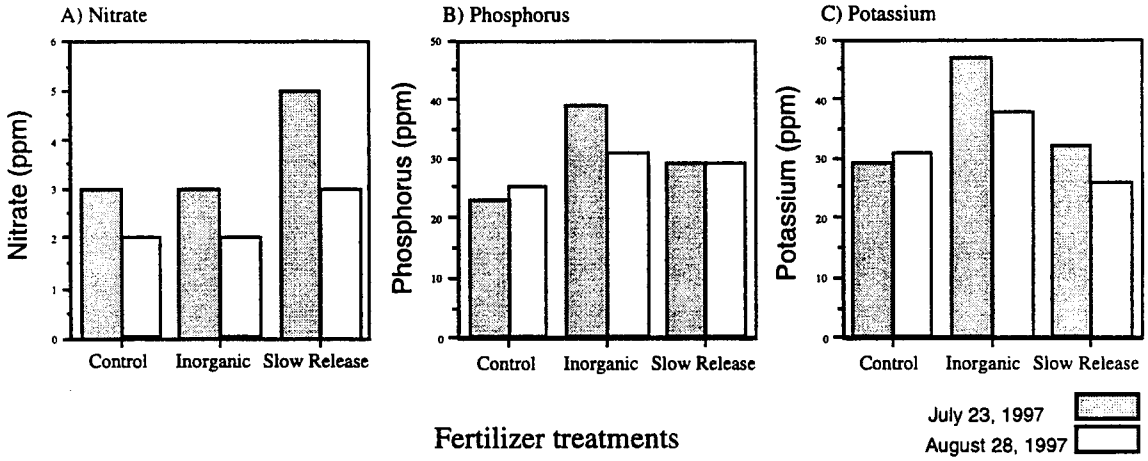

Figure 5.3. Soil nutrient level following restoration establishment. Soil samples were taken one month (gray bars) and two months (white bars) following fertilization and establishment of the restoration site. A) nitrate B) phosphorus (Olsen's) C) potassium levels in study plots.

5.3.2 *Soil nutrient levels in treated plots*

Nutrient analyses indicate that application of slow-release or inorganic fertilizer increased the level of nitrate or potassium respectively (Table 5.2). The increase in nitrate under the slow-release fertilizer regime was significant during the first season, and tended to be higher during the following two years. During the first two years of the experiment potassium levels were significantly increased in plots treated with inorganic fertilizer, and they remained high during the third year. Phosphorus levels were not significantly affected by the fertilization treatments,

however there was a noticeable trend towards increased phosphorus levels in fertilized plots, especially plots treated with inorganic fertilizer.

Table 5.2. Soil nutrient levels in fertilized plots. Numbers are nutrient levels (ppm)± SE. Letters following numbers indicate significant differences between treatments during the same growing season ($p < 0.05$).

	Nitrate (ppm)		
	1997	1998	1999
No fertilizer control	2.68 ±0.31 a	2.38 ±0.18	3.35 ±1.09
Inorganic	2.50 ±0.09 a	2.48 ±0.21	3.90 ±0.34
Slow release	4.65 ±0.72 b	3.50 ±0.56	4.93 ±0.69
	Phosphorus (ppm)		
	1997	1998	1999
No fertilizer control	22.63 ±0.99	23.00 ±1.00	21.50 ±0.96
Inorganic	26.50 ±1.66	29.50 ±2.06	29.75 ±2.47
Slow release	24.50 ±1.44	27.00 ±2.61	27.00 ±3.24
	Potassium (ppm)		
	1997	1998	1999
No fertilizer control	31.88 ±1.66 a	34.50 ±1.85 a	36.50 ±0.65
Inorganic	39.50 ±0.96 b	42.25 ±2.50 b	42.75 ±3.37
Slow release	28.00 ±1.08 a	34.13 ±0.43 a	38.00 ±3.19

5.3.3 *Plant community*

The number of plants counted in 1997, 1998, and 1999 were 14,286, 15,462, and 4,755 individual plants respectively. The predominant species in 1997 were weedy annual grasses; foxtail, barnyard grass, and crabgrass. Though present in high numbers in 1998 and 1999, these weedy annual grasses decreased in height, biomass, and seed set both years. Foxtail and crabgrass remained the predominant species in 1998. With a great deal of vegetative growth and many flowers on each plant, black-eyed Susan was a sub-dominant species in 1998. In 1999, the native grasses Indian grass, big bluestem, and little bluestem became the predominant species. Native forbs were also common in 1999 with wild bergamot, Canadian milk-vetch and black-eyed susan being present in many plots.

Of the 27 species planted, 18 were seen during the course of the experiment. Seed for switch grass (*Panicum virgatum*) did not grow in lab germination tests. All other grass seeds germinated in lab testing. Forb seeds were also tested in lab with satisfactory results, although germination of seeds requiring specialized treatments varied greatly and not all seeds germinated under our conditions.

Several unplanted desirable native species grew in and around the research plots (Table 5.3). The growth of unplanted natives at our site suggests that remnant populations from area roadsides, pastures, and remnant prairie stands are viable and being dispersed onto our site. Species found in or near our plots included swamp milkweed (*Asclepias incarnata*), evening primrose (*Oenothera biennis*), and hare figwort (*Scrophularia lanceolata*). The addition of unplanted natives served to invigorate the diversity of the native plant community.

Many undesirable species appeared in our study plots. Several of these were agricultural 'weedy' species such as ragweeds (*Ambrosia artimisiifolia* and *A. trifida*), leafy spurge (*Euphorbia esula),* lambsquarter (*Chenopodium album)*, and pigweeds (*Amaranthus* sp.) The majority of weedy forbs exhibited successional patterns and were not found in abundance for more than one season of the experiment. An example is horseweed (*Conyza canadensis*), which was found in great abundance only during the 1998 growing season. Some serious 'weed' outbreaks were noted during our study. Thistle species continue to be of concern and may outcompete natives in some plots. The weedy grass reed canary grass (*Phalaris arundinacea*) is also present on the site in at least one plot and appears to be slowly spreading.

Though many weeds were transient in nature, they dramatically affected some plots. For example, the dense cover provided by prickly lettuce (*Lactuca seriola*) on the southeast end of the field during the 1998 growing season limited growth of other species during that season and into the 1999 season.

Table 5.3. Plant species commonly found at the Shakopee research site.

Common Name	Scientific Name
Alfalfa	*Medicago* spp.
Barnyard grass	*Echinochloa crusgalli*
Big bluestem	*Andropogon gerardii*
Bindweed	*Polygonium* spp.
Black-eyed susan	*Rudbeckia hirta*
Canadian milk vetch	*Astragalus canadensis*
Canadian wild rye	*Elymus canadensis*
Cinquefoils	*Potentilla* spp.
Common evening primrose	*Oenothera biennis*
Common milkweed	*Asclepias syriaca*
Common mullein	*Verbascum thapsus*
Common sunflower	*Helianthus annuus*
Cow vetch	*Vicia cracca*
Dandelion	*Taraxacum officinale*
Foxtail	*Setaria* ssp.
Giant ragweed	*Ambrosia trifida*
Goatsbeard	*Tragopogon dubius*
Hare figwort	*Scrophularia lanceolata*
Hoary allysum	*Berteroa incana*
Horseweed	*Conyza canadensis*
Lambsquarter	*Chenopodium album*
Leafy spurge	*Euphorbia esula*
Ohio spiderwort	*Tradescantia ohiensis*
Pigweed	*Amaranthus* ssp.
Purple clover	*Trifolium pratense*
Ragweed	*Ambrosia artemisiifolia*
Reed canary grass	*Phalaris arundinacea*
Sedges	*Cyperus* spp.
Showy tick trefoil	*Desmodium canadense*
Sorrel	*Oxalis* spp.
Swamp milkweed	*Asclepias incarnata*
Thistle species	*Circium* and/or *Carduus*
White campion	*Silene pratensis*
White clover	*Trifolium repens*
White sweet clover	*Melilotus alba*
Wild Bergomot	*Monarda fistulosa*
Yarrow	*Achillea millefolium*

5.3.4 *Plant biomass*

Shoot biomass analysis showed that slow release fertilizer significantly increased plant biomass in the first and second season of the experiment (Figure 5.4A-C). During the third year, biomass in plots treated with the slow-release fertilizer regime appeared similar to control plots. The largest component of increased shoot biomass under the slow-release fertilizer treatment was undesired grasses, which were significantly higher than either of the other treatments for all three

years of the experiment (Figure 5.5). Plots treated with slow-release fertilizer also had significantly more undesirable forb biomass during the 1998 season than the other fertilizer treatments (Figure 5.6). Plots treated with inorganic fertilizer were not significantly different from fertilizer control plots throughout the experiment, nor were significant differences in biomass found among mycorrhizal treatments. However, during the 1999 season litter levels were found to be significantly altered by mycorrhizal treatments.

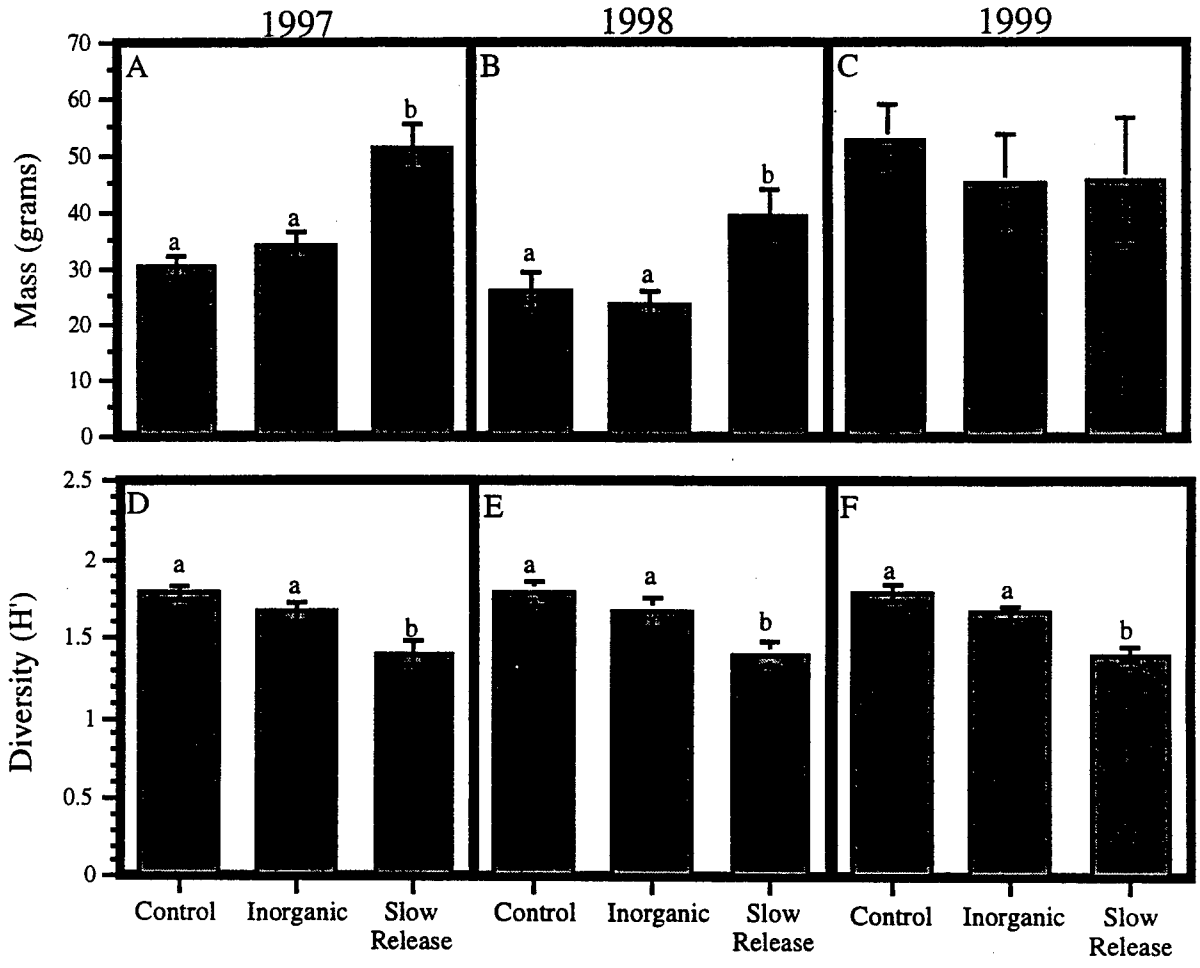

Figure 5.4. Total harvested biomass (A-C) and diversity (D-F) in fertilizer treatment plots for the years 1997,1998 and 1999 respectively. Error bars represent ± 1 SE. Within each panel, different letters indicate significant differences among treatments($p < 0.05$).

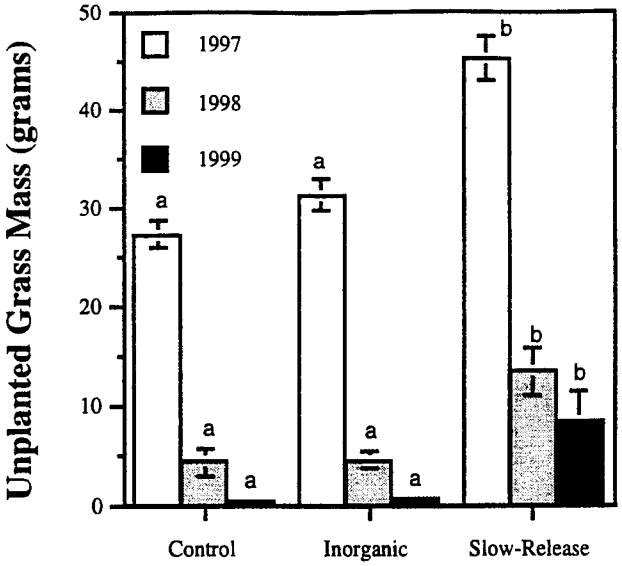

Figure 5.5. Mean (± 1 SE) above-ground biomass of undesired grasses in harvested 1000 cm^2 area under each fertilizer regime. Different letters represent significant differences among fertilizer treatments within each year ($p < 0.05$).

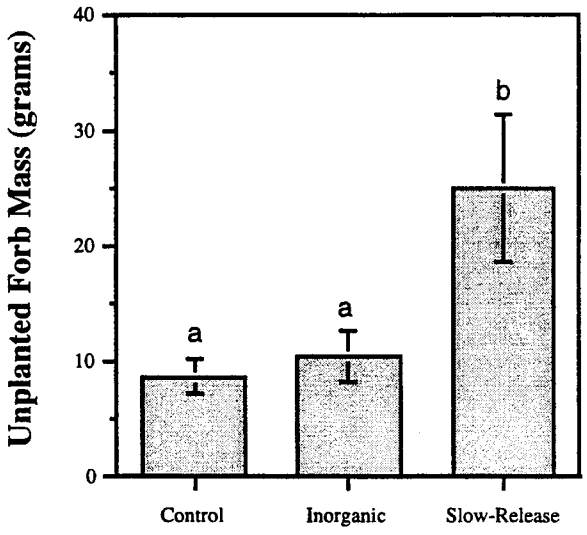

Figure 5.6. Mean (± 1 SE) aboveground biomass of undesired forbs in 1998. Different letters represent significant differences ($p<0.05$).

5.3.5 *Plant diversity*

Diversity, as measured by the Shannon-Weaver index, was significantly affected by fertilization treatments (Figure 5.4D-F). Significant reductions of diversity were seen in plots treated with slow-release fertilizer during all three years of the study. The decreased diversity of slow-release fertilized plots mostly reflected the decrease in grass diversity in slow-release treated plots (Figure 5.7). However, during 1999 the forb diversity was also significantly reduced in plots treated with slow-release fertilizer.

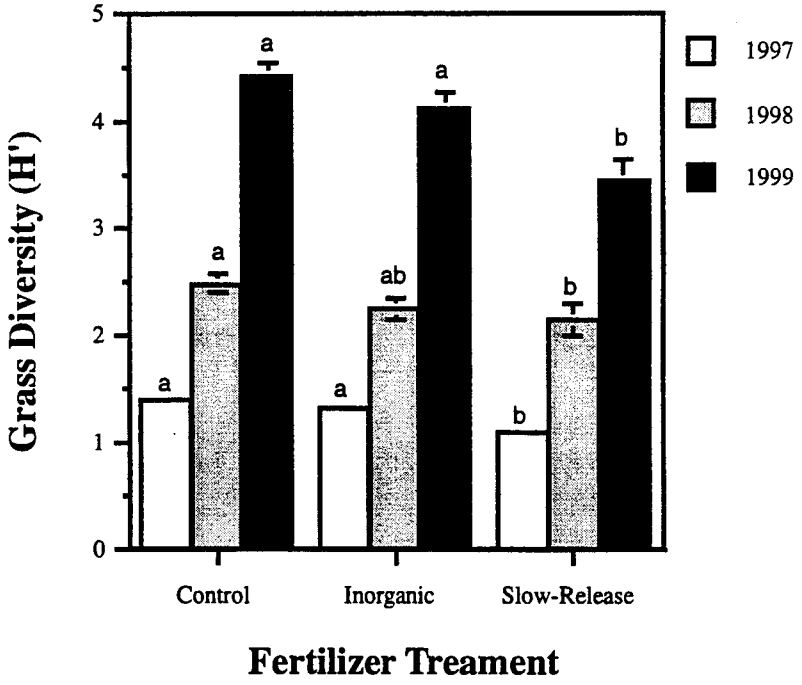

Figure 5.7. Mean grass diversity (±1 SE) in fertilizer treatment plots as measured by the Shannon-Weaver diversity index (H'). Different letters represent significant differences among fertilizer treatments within each year ($p < 0.05$).

A trend towards increased diversity was noted in some mycorrhizal treatments during the 1998 season however, it was not found to be significant ($p = 0.08$) (Figure 5.8).

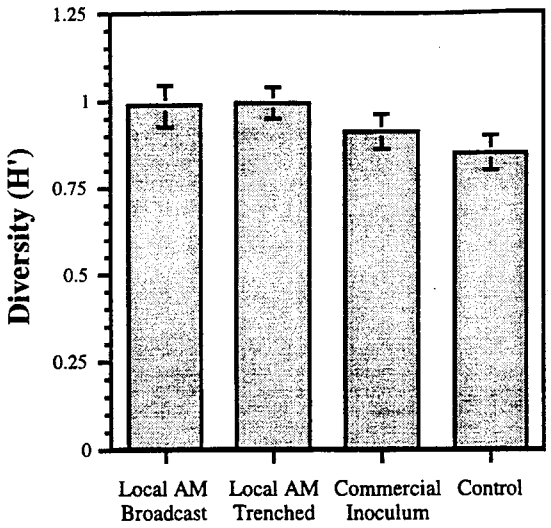

Figure 5.8. Mean (± 1 SE) diversity in mycorrhizal treatment plots in 1998 as measured by the Shannon-Weaver Diversity index (H'). Treatments were not significantly different (p = 0.08).

5.3.6 *Individual species response to treatments*

Individual species were analyzed for responses to treatments if they occurred in sufficiently high numbers to perform statistical analyses. Unfortunately, few species were found in high numbers in enough plots to perform these analyses.

The 1997 foxtail (*Setaria* spp.) data indicates that while the size of foxtail was increased by fertilization with slow-release fertilizer (Figure 5.9A), the number of foxtail plants per plot was reduced in plots treated with mycorrhizal amendments (Figure 5.9B). The 1997 barnyard grass data reveals it shares the same pattern of size and number with respect to fertilization and mycorrhizal amendment.

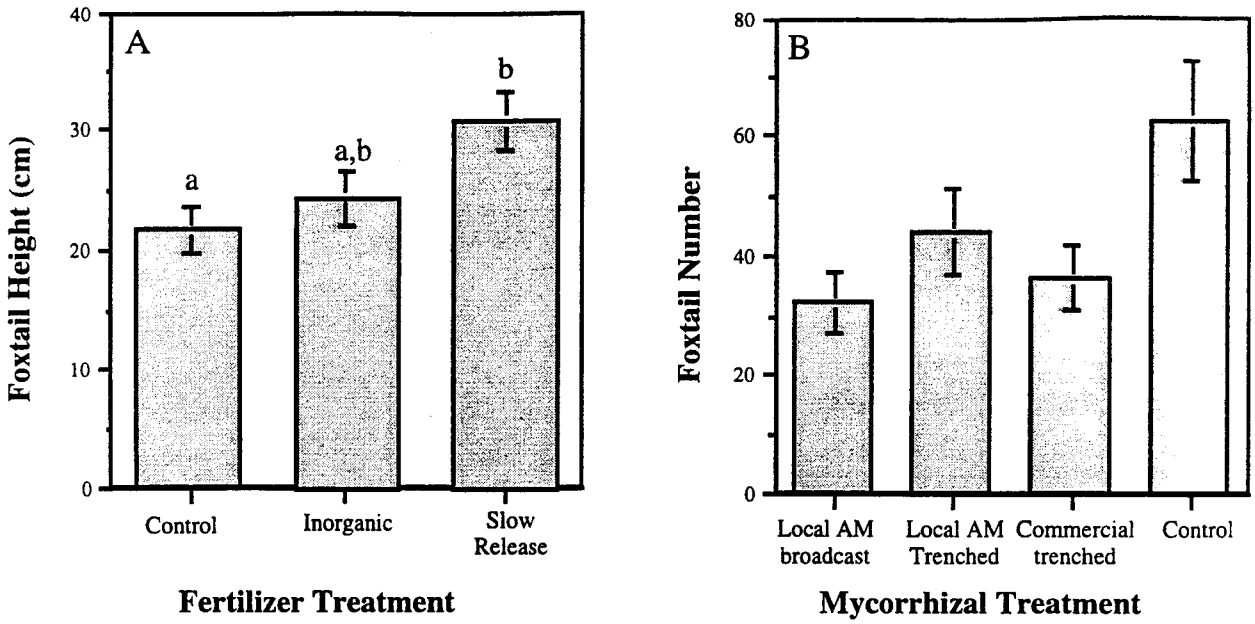

Figure 5.9. Mean height (± 1 SE) of foxtail plants in fertilizer treatment plots in 1997 (A). Mean number (±1 SE) of individual foxtail Plants in mycorrhizal amended plots in 1997 (B). Different letters represent significant differences between treatments ($p<0.05$)

5.3.7 *Mycorrhizal colonization*

In 1998, plots that received slow-release fertilizer had significantly higher levels of colonization than plots that received inorganic fertilizer, or plots that were unfertilized (Figure 5.10A). With regard to inoculation treatment, plots that received trenched inoculum, either commercial or lab produced, had significantly higher levels of colonization than uninoculated plots (Figure 5.10B). The broadcast lab-produced inoculum treatment was intermediate, and did not differ significantly from any other inoculation treatment. There was no evidence for interaction between fertilizer and inoculation treatments.

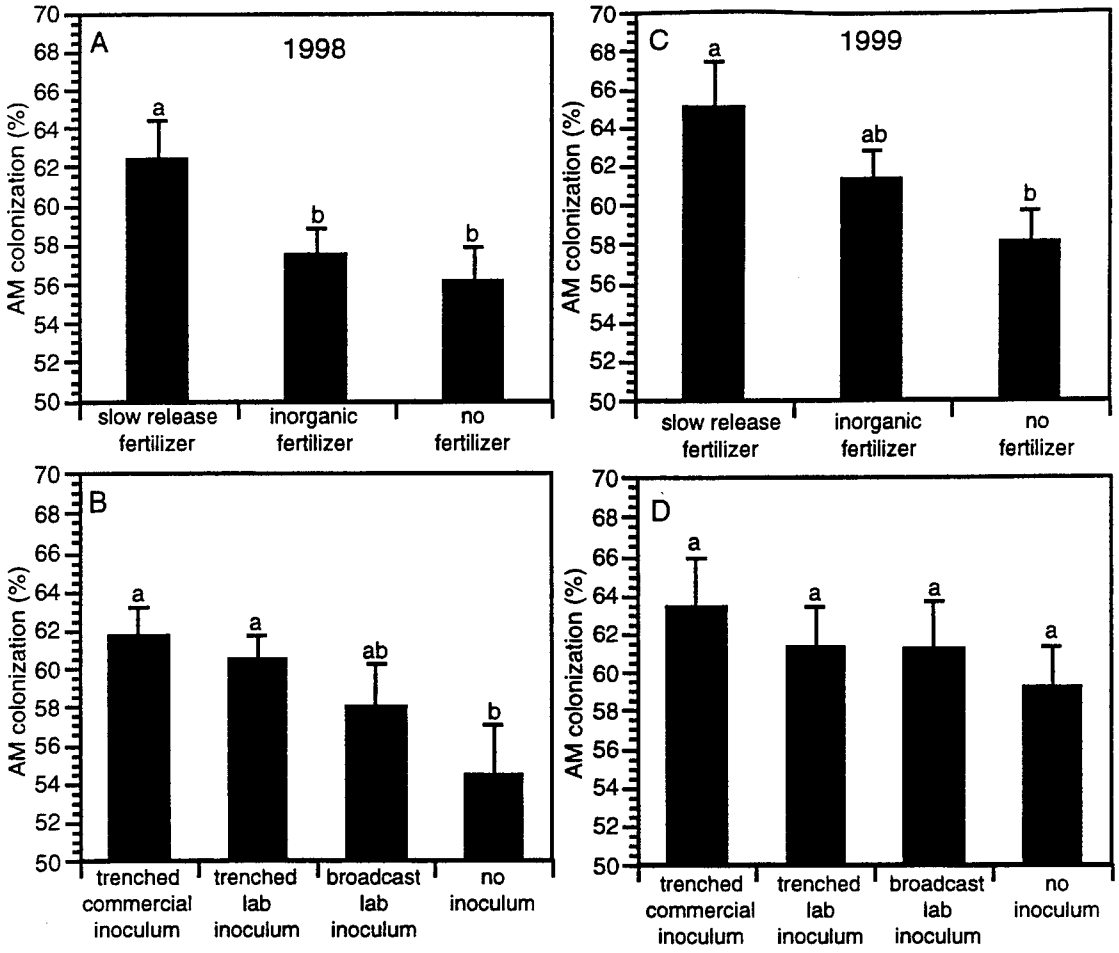

Figure 5.10. Mean (± 1 SE) AM colonization in 1998 (A-B) and 1999 (C-D) viewed by fertilizer or inoculation treatment. Within a panel, different letters represent significant differences among treatments ($p < 0.05$).

By 1999, average colonization levels increased in all treatments, but more so in treatments that had been lower in 1998; thus diminishing differences among the treatments. The slow-release fertilizer treatment still had significantly greater colonization than the unfertilized treatment, but was no longer different than the inorganic fertilizer treatment (Figure 5.10C). Likewise, there were no longer significant differences among inoculation treatments, although the pattern was consistent with that seen in 1998; higher colonization in the trenched commercial inoculum treatment, lower colonization in the uninoculated treatment (Figure 5.10D).

5.4 Discussion

Our results indicate that while mycorrhizal amendments affected some species of the restoration plant community, fertilization had far more influence on the plant community. Three years after a single fertilizer application, diversity remains lower in plots treated with slow-release fertilizer. Other studies on Minnesota prairies have shown that nitrogen fertilization affects diversity by promoting the persistence of weedy annual species [49]. Native plant communities are adapted to low nutrients and appear to not benefit from fertilization.

Although slow-release fertilizer was used to test a continual low-level nutrient application targeting native plants, it appears that the main beneficiary of these nutrients was weedy annual grasses. Their increased size and abundance in slow-release fertilized plots appeared sufficient to limit other species and resulted in lower diversity for these plots. Crabgrass data indicates that in plots treated with slow release fertilizer, the number of crabgrass and their size was reduced. The most likely limitation for crabgrass was a reduction of available light, as the larger foxtail and barnyard grass occupied much of the canopy area in slow-release fertilized plots

Vegetation and AM fungal colonization in mineral fertilizer treated plots were not found to be different in any way from our control treatments. A possible explanation is that the mineral fertilizer, especially the nitrate component, dissolved during heavy rains early in the experiment and moved by bulk flow with the water table to the adjacent wetland. The use of easily dissolved mineral fertilizers in roadside planting is questionable, as one of the purposes of roadside areas is the conveyance of water away from the roadway surface. Ditches and roadsides are often moist and can for long periods of time be 'mini-wetlands', indicating that highly soluble fertilizers should not be applied.

Mycorrhizal amendments appeared to have had a limited impact at this site. While individual species might be sensitive to mycorrhizal amendment, global parameters such as aboveground biomass were not affected by mycorrhizal treatments. Several potential explanations exist for the lack of plant community differences among the mycorrhizal treatments. One possibility is that the observed increase in mycorrhizal colonization in inoculated plots was not sufficient to elicit a response in the plant community. Average colonization was 10-12% higher in the trenched inoculum plots than the uninoculated control in 1998. While statistically significant, it's possible that this was not a difference sufficient for

biological significance, given that uninoculated plots had ~50% colonization. It's also possible that any resultant shifts in plant community parameters were too subtle for us to detect.

However, Smith et al. [5] had comparable differences in colonization among inoculation treatments, and observed significant responses in the plant community. It is likely that the contrasting results between these two studies arose from differing baseline colonization levels between the two sites. Prior to inoculation, Smith et al. found essentially 0% colonization at the Cambridge, MN study site. In contrast, previous work at the present study's Shakopee site indicated that a population of mycorrhizal fungi was already present at the site (albeit at a relatively low level) prior to implementation of this study (unpublished data). It is likely that natural recolonization of the site by local remnant AM fungal populations was rapid and sufficient to colonize all plots. Additionally, tillage of the site prior to the current restoration may not have destroyed all fungal propagules and remaining AM fungal propagules could have quickly colonized vegetation in our plots.

We may have achieved greater plant community and AM colonization differences among inoculation treatments had more inoculum been used per plot (Table 5.4). The inoculum dosage used was established based on the work of Noyd et al. [4] and Smith et al. [5], who used 1 kg (2,140 spores) and 0.875 kg (33,250 spores) per square meter respectively (Table 5.4). Using 3 well-mixed batches of lab produced inoculum, we applied 0.635 kg (24,130 spores) per square meter. Commercial inoculum was applied at a rate of 0.146 kg/m^2, which corresponded to 4,305 spores/m^2 according to the manufacturer's literature. Little work exists on application of mycorrhizal inoculum in systems other than agriculture, and even in agricultural studies, the number of propagules needed to produce an observable effect varies greatly. Therefore, it is difficult to predict whether addition of a higher number mycorrhizal propagules would have produced a more observable response. However, given the cost of inoculum, it seems unlikely that such an increase would be practical or desirable, particularly when there is an extant mycorrhizal population in the soil.

Table 5.4. Mycorrhizal application rates in prairie restoration studies.

Application	Current Study			Smith	Noyd
Rate and Method	Trenched	Broadcast	Commercial	Trenched	Broadcast
Spores per Plot	96529	96529	17223	66500	21400
Spores per Sq. M	24132	24132	4305	33250	2140
Kg per Sq. M	0.635	0.635	0.147	0.875	1.00

Testing of the seed and inoculum application methods was conducted to examine whether a concentrated row of AM inoculum applied with seed was more beneficial in producing a healthier native plant community than a less concentrated dosage of inoculum seeded randomly over the entire plot. We found that both the broadcast and 'drilled' seed-row methods of seed application gave comparable results in terms of vegetation. Our results suggest that these methods can both be used effectively during restoration and should be selected based on site conditions and available seeding resources. In terms of mycorrhizal colonization, we found that broadcast application resulted in somewhat lower levels of AM colonization, consistent with a number of published studies [55]. However, given the absence of mycorrhizal effects on the aboveground plant community, this contrast becomes largely irrelevant in terms of vegetation establishment.

Though not tested against plots receiving the forb seed mix specified by Mn/DOT (2% forbs by seed weight [8]), our high forb mix (10%) plots had what we considered to be a larger abundance of forbs. However, many of the forbs present were one of the three species; Canada-milk vetch, black-eyed susan, or wild bergamot. Several other forb species were found in low numbers, but not readily observable under the canopy of the dominant forbs. In analyzing the seed mix specified by Mn/DOT, we found that specifying the same weight (0.05 kg/h) for all forbs in the seed mix led to very high numbers of some lightweight seeds and low numbers of heavy seeds. As an example, planting 0.25 kg/h of butterfly milkweed (4,300 seeds per ounce) versus 0.25 kg/h blue vervain (93,000 seeds per ounce) yields 3.8 seeds/m^2 of butterfly milkweed as compared to 82.2 seeds/m^2 of blue vervain. Though convenient in specification of seed mixes, using the same weight for all species of forbs most likely does not result in the balance of forbs found in nature nor the desired amount of certain species. An alternative method of designing seed mixes, described by Dibol, uses the desired seeding density (seeds per hectare) to calculated the seed mix [56]. In combination with available seed mass data [57], this method would allow vegetation managers to better tailor the forb component of seed mixes and could be used in conjunction with seed prices to select reasonably priced, diverse seed mixes.

The success of the seed mix planted at our research site was very good in light of high water table and soil moisture found on the site. The seasons preceding our work were relatively dry and led us to believe that this site should be planted with a mesic prairie mix. Our mesic seed mix grew very well during three seasons in which the water table was very close to the

surface. Successful growth was most likely due to the very sandy soil, which provided excellent drainage and resisted waterlogging.

The high water table early in our experiment led us to speculate on possible refinements of seeding methods for sites with unknown hydrology, such as ours. One suggestion was to plant the native grass component immediately after landscaping and grading has finished and allow a few seasons to pass before selecting and planting a forb mix. The delayed planting would allow the site hydrology to be monitored for multiple seasons before the more expensive forb seed mixture is planted. An alternative seeding method would be a contoured planting, which has xeric and mesic seed mixes planted in elevated areas and wet mixes in the low-lying areas. Both methods require additional work and expense, yet they have the potential to reduce restoration failures or poor revegetation and thus save the expense of increased maintenance or replanting the site.

Though we have suggested possible improvements of Mn/DOT's seeding methods, the success of our restoration using Mn/DOT's protocols demonstrates that the current methods work well. Precisely following the guidelines of the Minnesota Department of Transportation Seeding Manual 1996/97[8], we established a vigorous prairie plant community with a good balance of forbs and grasses. In using these guidelines, we paid particular attention to purchase of quality seed from reputable local vendors, proper site preparation, and planting seed in accordance with Mn/DOT suggested seed depth. Our suggested improvements are given to improve the current guidelines and widen the scope of native plantings that Mn/DOT can conduct during their work.

5.5 Conclusions

Though useful in producing more plant biomass, fertilization does not appear to benefit native prairie plantings. Our study and others reveal that nutrient addition lowers diversity and favors undesirable 'weedy vegetation'. In addition, many Mn/DOT sites are prone to standing or flowing water, which would tend to leech fertilizer into the watershed.

In situations where erosion is a factor and quick revegetation is required, slow-release fertilization use might be warranted. However in these situations, non-native plants could perhaps provide the best immediate cover, with a later inter-seeding of natives into the established non-native stands.

Though mycorrhizal amendments have demonstrated the ability to enhance plant cover at restoration sites, their overall impact on improving restoration success has been minimal in our studies. A thorough cost/benefit analysis of mycorrhizal amendment treatments should be conducted before their widespread use in restorations. The analysis should take into account several factors, among them; prior vegetation history, nutrient availability & soil type, remnant or local populations of mycorrhizal fungi, and the need for quick plant establishment.

One estimate placed the cost of inoculum amendment at between two hundred and several thousand dollars per hectacre [44]. Using the commercial inocula we selected at the manufactures suggested rate, field application would cost an estimated fifteen thousand dollars per hectare. However, future advances in the field of mycorrhizal inoculum production may lower the cost of producing such inoculum and therefore make it more cost effective to apply at restoration sites.

Sites likely to benefit from mycorrhizal amendments are those that have been extremely damaged and lack most biological activity such as mine reclamation sites. Mine sites often have few remnant mycorrhizal propagules, very nutrient poor 'soil', and little topsoil. The difficulties in establishing vegetation on the nutrient poor, sometimes alkaline, erosion prone mine-tailings could justify the expense of mycorrhizal amendments, but should be decided on an individual basis.

Less disturbed areas are not as likely to benefit significantly from mycorrhizal inoculation. In these areas, remnant mycorrhizal populations are more likely to be present and can serve as a source of inoculum. Other near-by populations of mycorrhizae may also be present to supply AM propagules, even at highly disturbed reclaimed mine sites. For example, Noyd et al. [4] found that three years after reclamation, the amount of AM fungi in un-inoculated taconite plots was equal to those to which inoculum had been applied.

Financial and other resources used for fertilizer and mycorrhizal amendments may be better used in other areas of a restoration project. Increased resources might be shifted towards site preparation, to reduce weeds prior to restoration or provide native plants or seeds with a better seedbed. Another use for increased resources might be in maintenance regimes such as controlled burns or mowing, which may be of more value than soil amendments.

Based on our use of the higher forb seed mix, it would be beneficial to include a higher percentage of native forbs in the seed mix. Alternate methods of designing seed mixes may also

provide better control of forb density and diversity (see above). Targets of forb density and diversity at restoration sites should be generated to determine whether the amount of forb seeds in Mn/DOT specified seed mixtures is at an appropriate level.

5.6 Recommendations

1) Fertilization should not be used on prairie restoration under most circumstances. Exceptions to this might include cases where rapid vegetation establishment is needed, sites that have extremely low nutrient availability, or sites prone to erosion. In these cases, slow-release fertilizer should be used to avoid leeching of nutrients into the local watershed.
2) Our study examining typical horticultural doses of nutrients during prairie restoration revealed significant negative effects; however, further work is required to establish whether lower doses or other forms of nutrient amendment, would benefit native plantings.
3) Sites undergoing prairie restoration should, as a regular practice, be analyzed for nutrient levels, pH, and organic matter prior to restoration. Using site specific information, soil can be amended as needed to permit the best possible growth of the desired species without wasting unnecessary or deleterious resources.
4) Mycorrhizal amendments may benefit highly degraded restoration sites but may not be cost effective at most typical restoration sites. A cost/benefit review should be conducted in the future if inocula costs are dramatically reduced.
5) Increasing the amount of forb seeds applied during restorations and reclamations would benefit the native plantings and provide prairies more closely resembling remnant native prairies
6) Native prairie restoration using the protocol established by Mn/DOT was highly effective in establishing native plants which should with limited management become a self-sustaining native plant community.
7) Seed mixes should be assembled based on seed number per unit area, rather than seed mass per unit area.

8) Though regional seed mixes designed by Mn/DOT offers some variation in species, site-specific seed selection should be considered during the planning phase of restorations.

9) Hydrology on potentially hydric restoration sites should be monitored prior to planting expensive seeds in order to tailor seed mixes to given sites.

Chapter 6. The influence of maintenance techniques (burning, mowing) on mycorrhizal colonization and the prairie plant community

6.1 Overview

Tallgrass prairie was once a dominant vegetation type in the state of Minnesota. The success of the diverse plants within this community was supported largely through the action of periodic fires. However, beginning with the period of intense European settlement in the mid-nineteenth century, fire has been excluded from most prairie areas. In conjunction with other human-related factors, such as the turnover of lands to agriculture and habitat fragmentation, fire suppression has led to a sharp decline in extant prairie lands [58]. Now, only scattered remnants of the tallgrass prairie still remain and many of the existing grassland areas are dominated by weedy or exotic plant species.

Recognition of the importance of tallgrass prairies to the ecology and natural history of Minnesota has prompted increased attempts to restore prairies throughout the state. The Minnesota Department of Transportation is one of many organizations that have begun to designate funds and research efforts towards delineating a management strategy for restoring and maintaining tallgrass prairie. Mn/DOT is responsible for the maintenance of extensive tracts of roadside grassland in Minnesota. However, little is known regarding methods for removing exotics, establishing native species, and maintaining prairie plant communities in roadside areas. Establishing a management program that promotes self-sustaining prairie vegetation, as well as one that maximizes the efficiency and effectiveness of prairie managers, is one of the most important goals in current prairie research.

Restoration is the re-establishment of the historic structure and function of an ecosystem. Since fire was once a key factor in the maintenance of both the structure and the function of tallgrass prairie vegetation, controlled burns are commonly included in any prairie restoration plan. However, the use of fire in roadside areas is often not feasible, due to dangers from smoke on the road or uncontrolled spread of the burn. Therefore, restoration of roadside areas is further complicated by a need to replace burning as a periodic disturbance.

Fire served a number of key functions in grasslands. It prevented the growth of woody species and thereby inhibited the process of succession [58, 59, 60, 61]. Burning promoted new seedling growth and served as a form of disturbance and competitive release so that new plants could initiate growth, preventing dominance by a few successful species [62, 63]. Furthermore,

many species of plants in different regions of the prairie have shown an increase in cover and productivity following a fire [64, 65, 66]. Finally, fire has been shown to be detrimental to the growth of some species of exotic grasses [67, 68, 69, 70, 71, 72, 73]. Any management strategy that was going to serve as a replacement for fire would have to be able to support these same types of changes in the prairie.

Research suggests that mowing can have a similar effect as burning on the prairie plant community [9, 10, 11, 12] Mowing of roadside areas has been done extensively in the past, but more commonly as a part of a maintenance plan rather than as part of a restoration strategy. The exact impact of annual mowing on restored prairies is still unclear; although some researchers have found that native plant diversity or cover increases in response to mowing treatments [74, 75]. It is possible that mowing along roadside areas could reduce cover of exotic species and promote increased cover and diversity of native vegetation.

It was the goal of this research to monitor the effect of burning on the vegetation, soil properties and myccorhizal colonization of plants in roadside areas in Minnesota. Roadside restoration sites in St. Cloud and Cambridge were evaluated following a burn. Soil parameters, including pH, organic matter, and nutrient status were monitored immediately after the fire in St. Cloud to evaluate changes caused by the burn as well as to determine site characteristics along a continuous stretch of highway. Both diversity and cover of native species were measured at the St. Cloud site to determine the effect of an early spring fire on individual plant species and the overall plant community composition in the year following the burn. Additionally, some plots in St. Cloud were mowed in July 1998 and the vegetation was left on site. This treatment mimics one of the many possible mowing treatments that has been used by Mn/DOT to control the vegetation in roadsides. The strategy of mowing later in the growing season and adding to the litter layer would likely not provide the same advantageous effects that fire might have on the native species. By combining mowing and burning treatments, we could investigate the effect that maintenance mowing could have on attempts to restore prairie structure and function by burning. We hoped to determine if burning is an effective strategy for creating and maintaining native plant populations while at the same time decreasing the cover of unwanted exotic plant species. Furthermore, the research at Cambridge studied the impact of a summer burn on soil nutrient properties, level of mycorrhizal colonization in plant roots, and flowering of big bluestem. With this research, we hoped to pinpoint some of the changes that occur within the

soil and the plants after a burn to identify the mechanism via which fire affects the productivity of prairie plants.

6.2 Prairie maintenance techniques at TH15, St. Cloud

6.2.1 *Materials and methods*

Study area:

Study plots were established along the east side of Trunk Highway 15 (TH15) south of the city of St. Cloud, Minnesota in Stearns County, 45.5°N and 94.16°W. This stretch of TH15 extends from interstate highway 94 to the western edge of the St. Cloud downtown area. Following construction work on TH15 in 1991, the Minnesota Department of Transportation seeded the right-of-way area. Mn/DOT has been responsible for the maintenance of these areas since that time. In general, the land immediately adjacent to the road slopes down, but the plots were located in the relatively flat land approximately 10 meters from the edge of the pavement. Exotic grasses and forbs dominated the roadside plant community, including smooth brome (*Bromus inermis*), Kentucky bluegrass (*Poa pratensis*) and birdsfoot trefoil *(Lotus corniculatus*). Prairie species were unevenly distributed throughout the right-of-way. Native grasses found on site included big bluestem (*Andropogon gerardii*), little bluestem (*Schizocarium scoparium*) and Indian grass (*Sorghastrum nutans*). The most abundant native forb was bergamot (*Monarda fistulosa*) but other species, including some composites, asters, and native clovers, were also present. Soils in this region are generally classified as fine loamy sands. St. Cloud is located within the Anoka Sand Plain region of Minnesota.

Experimental design:

Five blocks of four plots each were established on April 24, 1998 after Mn/DOT had burned selected quarter-mile stretches of right-of-way area. The blocks were disjointed in space, as they were established after the burn and designed to straddle burned/unburned borders. Each block had two plots in the burned area and two plots in the adjacent unburned area. Block 1 was positioned by itself on the edge of a small burn. Blocks 2 and 3 were at the northern and southern ends (respectively) of the same stretch of burned area. Blocks 4 and 5 had a similar configuration in the next burned area. The distance between plots within a block was 5m, except in Block 4, where the border between the burned and unburned areas was very uneven. Therefore, there was a 10m gap between plots in the burned and plots in the unburned area.

Each plot was 10m x 10m, but samples of soil and vegetation were taken from a central 5 x 5m area within each plot to avoid edge effects. All plots were positioned so that their eastern border was 1m away from the fence that runs parallel to the highway.

Mn/DOT mowed two plots within each block on July 22, 1998. The cut vegetation was left on the plots.

Sampling:

Soil samples were taken in all 20 plots on April 24, July 24, and October 2, 1998. The cores were immediately bagged, placed on ice and taken back to the lab. They were stored in the cold room at 4 °C before being sent to the University of Minnesota Research and Analytical Laboratories for analysis. Samples were evaluated for pH, potassium, nitrate, phosphorus (using either the Bray or Olsen tests depending on sample pH), and organic matter content.

The vegetation sampling was performed on May 27, July 27, and August 31 in 1999 using the point-frame method developed by Goodall [76]. The frame was one meter long, with ten pins evenly spaced along its length. The frame was set down at ten random locations in each 5 x 5m plot. Each time a leaf, flower, or stem came into contact with one of the ten pins, it was counted, even if there was more than one contact point by the same individual plant. Dead vegetation was not included in the counts. Total counts for each plant species were totaled for each plot, providing an estimate of plant cover for every plot. This method often results in counts exceeding 100% because the overlapping layers of vegetation are taken into account.

Analysis:

Systat v. 9.0 was used to perform statistical analysis on soil parameters and vegetative percent cover. Variance between blocks and between mowing and burning treatments was evaluated for pH, phosphorous, potassium, nitrate, and organic matter. Additionally, an analysis of variance was done for percent cover of native and non-native species from each of the three sampling dates for both mowing and burning treatments.

6.2.2 *Results and discussion*

Soil:

Soil was analyzed for pH, organic matter, nitrate, phosphorus, and potassium concentrations (Table 6.1). Statistical analysis showed that there was a significant difference in

pH between blocks for all three sampling dates ($p < 0.001$). Since ash is known to be basic, it was expected that the pH of soil in sites that had been burned might increase slightly after treatment. However, there was no significant treatment effect on pH noted in this experiment. Analysis also showed that there was significant difference in nitrate, potassium, phosphorus, and organic matter between the two groups of blocks for each sampling date. Differences in the nutrient levels within the five blocks might be related to the pH differences.

Table 6.1. Soil Characteristics for plots along Trunk Highway 15 in St. Cloud, MN, April 24, 1998.

Block	pH	Organic matter (%)	Nitrate (ppm)	Phosphorus (ppm)	Potassium (ppm)
1	$7.30^{a1} \pm 0.24$	$2.20^{a} \pm 0.29$	$13.04^{a} \pm 2.44$	$26.38^{a} \pm 2.87$	$43.50^{a} \pm 11.31$
2	$6.35^{b} \pm 0.16$	$3.10^{b} \pm 0.14$	$15.48^{a} \pm 1.55$	$39.00^{b} \pm 4.22$	$103.50^{b} \pm 19.14$
3	$7.80^{a} \pm 0.04$	$1.53^{c} \pm 0.11$	$7.73^{b} \pm 1.48$	$22.50^{a} \pm 1.26$	$45.00^{a} \pm 6.10$
4	$6.70^{b} \pm 0.09$	$3.10^{b} \pm 0.12$	$23.28^{b} \pm 2.10$	$31.50^{a} \pm 3.23$	$87.75^{c} \pm 6.05$
5	$6.63^{b} \pm 0.17$	$3.28^{d} \pm 0.14$	$14.43^{a} \pm 0.94$	$24.25^{a} \pm 1.75$	$79.25^{d} \pm 6.73$

[1] Means with different letters were significantly different at the $p < 0.05$

The results do not indicate a significant treatment effect on phosphorus values. Potassium concentrations were significantly lower in Blocks 1 and 3, which also had the lower pH. Again, there was no significant treatment effect on potassium values.

The nitrate concentration showed a significant response to the burning treatment in July (Figure 6.1). Sites that had been burned in early April of that year had significantly lower nitrate values than unburned plots. This difference had disappeared by the October sampling point. The variation over time of nitrate levels in burned plots was also significant, as amounts of NO3 dropped sharply between the April and July sampling dates.

The change in nitrate concentration in the burned plots at TH15 appears similar to the response seen in the soils at JES (see below). Nitrate levels peaked 14 days after the fire in plots that had been burned and then had decreased to levels similar to unburned plots by the end of the summer at the Cambridge site. The first nitrate samples taken at TH15 occurred approximately two weeks after the fire. Therefore, the high nitrate values in these first samples from burned plots could correspond to the high nitrate values found at JES two weeks after burning. Changes in nitrate in the soil following a fire is likely not due to inputs from the ash because dead prairie

vegetation is typically low in nutrients [77]. It is more likely that the nitrogen pulse after a fire is due to the impact of some other factor, such as increased rate of infiltration of inorganic N from rainfall [78]. Therefore, the same impacts on N levels that are created by fire could also be created by a mowing treatment when vegetation is removed. Researchers have found that adding N fertilizer to unburned plots did not have a significant effect on the production of big bluestem [79] indicating that changes in N levels are not one of the factors that stimulate increased plant production following a fire.

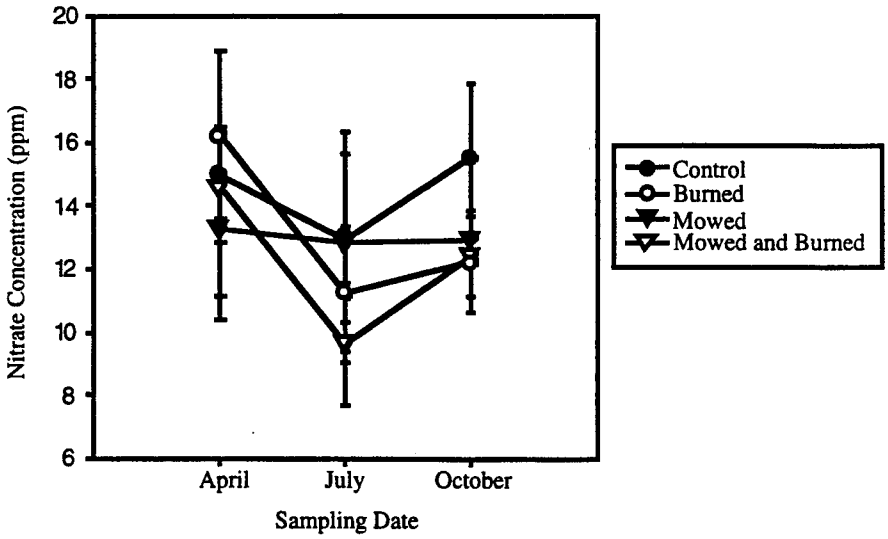

Figure 6.1. Soil nitrate content in response to treatment for three sampling dates at five sites along Trunk Highway 15 in St. Cloud, MN.

Organic matter concentrations in soil did not display any response to treatment. Multivariate analysis showed that there was a significant increase in percent organic matter in the soil with time. This was an expected result as the litter layer begins to accumulate and break down during the summer months.

The variation in pH and other soil properties along a continuous stretch of right-of-way suggests some of the difficulties in working with roadside prairies. These variations in soil quality might make it more difficult to create a management plan that will benefit the vegetation at all locations within a roadside area. If different soil characteristics cause the vegetation of a site to respond differently to treatment, should appropriate management strategies be established for individual sections of right-of-way? This process would likely be costly. However, new management strategies and technology make micro-scale management of a variable site a

possibility for the near future. Hopefully, in the future such management plans will produce beneficial results over a variety of soil nutrient properties. Additionally, if greater consistency in soil characteristics within the same roadside areas could enhance the success of restoration attempts, then Mn/DOT could include this as a management priority.

Vegetation:

There were no significant differences in total native species cover with treatment or with block seen one year after the burn event at TH15. However, total cover of natives did increase with time (Figure 6.2). Average cover increased from 9.70% on the May sampling date to 55.25% on the August sampling date summed over all plots and treatments. The five control plots had the lowest mean percent cover of native species on both the July and August sampling dates but this difference was not significant. In general, native cover was low in comparison to total cover of exotics. The highest values for percent cover of natives were in the low 70's and never exceeded 75%; whereas exotic species cover was always greater than 150%.

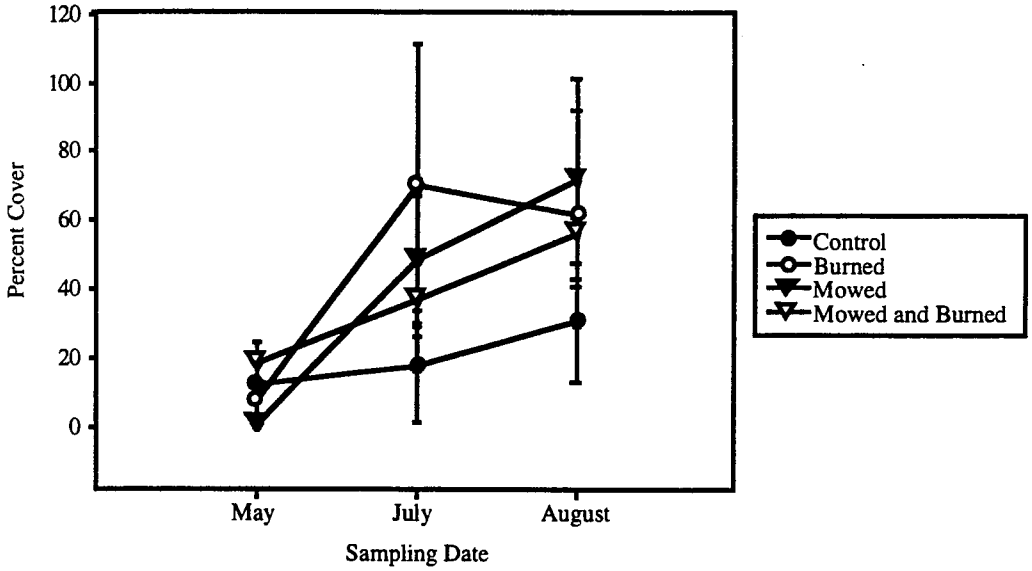

Figure 6.2. Percent cover of native species in relation to treatments for three sampling dates at five sites along Trunk Highway 15 in St. Cloud, MN.

Other researchers have found that total cover of native species increases in response to an early spring burn. In a southern Minnesota prairie, big bluestem cover increased three-fold after 13 years of annual burning [65]. Data from Hulbert shows that big bluestem production increased 151% and that the number of flower stalks increased 435% after a fire on a Kansas

tallgrass prairie. However, both of these studies were conducted on restored prairies that had a healthy established standing crop of big bluestem or other native species. In an extensive literature review, no studies were found that evaluated the ability of fire to increase native cover on plots that were heavily dominated by weedy species. Mowing has also been shown to increase the cover of native species, especially on new restoration sites [75]. Mowing removes canopy dominants and allows young seedlings to access resources such as light. Many native species will continue to respond positively to occasional mowing events after they have become established [11, 12, 66].

Exotic species have been shown to respond negatively to fire. Curtis and Partch [67] reported that bluegrass production was significantly less on plots that had been burned than on unburned sites. In a restored prairie in Wisconsin, Henderson [80] found that bluegrass disappeared entirely from plots that were burned for nine consecutive years. Mowing has also been shown to decrease the productivity of exotic grasses [11, 81, 82]. Along TH15, neither a burning nor a mowing treatment had significant effects on percent cover of exotic species (Figure 6.3). There was a trend towards higher total cover of exotics on control plots but it was not significant.

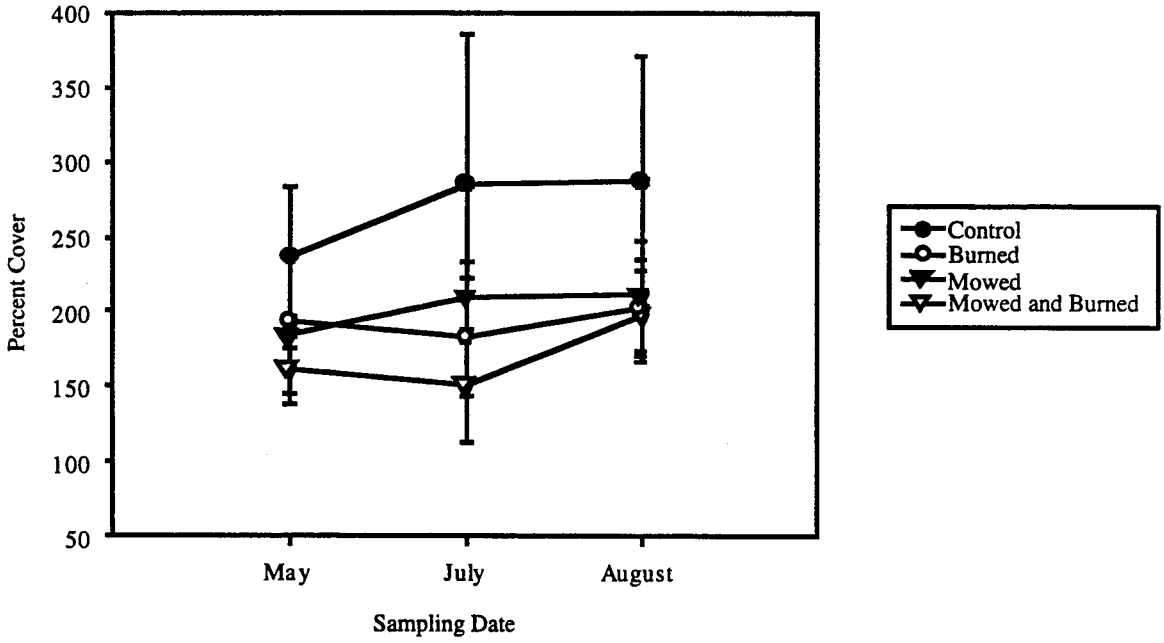

Figure 6.3. Percent cover of exotic species in relation to treatment for three sampling dates at five sites along Trunk Highway 15 in St. Cloud, MN.

The roadside areas that were studied in this research were planted in the early 1990's. It wasn't until 1998 that the first attempts were made to restore the native structure and function to these prairie sites. Due to the eight year lag between planting and monitoring, the research plots were overrun with exotic species when the first restoration management strategy was implemented. The non-native species found along TH15 are extremely difficult to eradicate once they have become well-established and can out-compete many of the natives for resources. The success of any restoration is severely hampered by the presence of these exotic species. Both of the management treatments utilized in this study did not have any significant effect on the cover of exotic vegetation in roadside plots. It is likely that a greater investment of energy during the early stages of ecosystem development could lead to higher diversity and cover of desired native species while at the same time minimizing the abundance of exotics.

6.3 Experimental burn at JES

6.3.1 *Materials and methods*

In 1998, Amy Moore, an NSF Aquatic Environmental Sciences Program intern, undertook a small scale burn experiment at the JES restoration area near Cambridge, Minnesota, at the intersection of highway 65 and county road 30 (see Charvat et al 1998 for further site description). In 1995, following construction, prairie grasses and forbs were planted in an approximately 15 meter wide strip alongside country road 30. For this study, six 10×10 meter square plots were set up in this strip of restored prairie. On July 9, 1998, three consecutive plots were burned and the other three plots were left unburned. Soil samples were collected at five times: 8 days prior to burning (pre-burn), 1 day post-burn, 2 weeks post-burn, 4 weeks post-burn, and 6 weeks post-burn. Additionally, Clarence Jackson, an NSF intern the following summer, continued the experiment, and collected soil samples on 6/22/99, corresponding to approximately 1 year post-burn.

Portions of all soil samples were frozen until sent to the University of Minnesota Research and Analytical Laboratories for quantification of soil phosphorus, ammonium and nitrate. In 1998, the remaining soil from the pre-burn, 1 day, 2 week, and 4 week post-burn samples was used to estimate mycorrhizal colonization. Roots were isolated from the soil samples, stained with 0.05% trypan blue in lactoglycerol [modified from 40] and mounted on slides to determine percentage colonization using the magnified intercept method [21].

Percentage colonization measures the percentage of intersects containing vesicles and/or arbuscules. In 1999, mycorrhizal colonization was analyzed in a like manner, but only vesicular colonization is presented.

Finally, as an estimate of the effect of burning on plant reproductive allocation, the number of big bluestem (*Andropogon gerardii*) inflorescences per plot was counted on July 19, 1999. At this time, many reproductive stems from the previous year were still attached, and were counted as well. While probably an underestimate of total reproductive output, the 1998 infloresence data can still give evidence of large differences in big bluestem reproductive activity following the burn.

6.3.2 *Results and discussion*

Overall, phosphorus levels were relatively constant in both the burned and unburned plots, but were significantly higher in the burned plots ($F = 39.1$, $p = 0.003$; Figure 6.4A). However, because the initial, pre-burn phosphorus levels were marginally higher in the burn plots as well ($p = 0.09$), it seems inappropriate to attribute this difference to the burn treatment.

In contrast, ammonium levels showed a distinct seasonal response: at the four week sampling point (8/6/1998), ammonium levels dropped by almost half in both the burned and the unburned plots ($F = 44.564$, $p < 0.001$). Ammonium levels were not significantly different between the burned and unburned treatments ($F = 4.687$, $p = 0.096$), although there was a trend toward higher values in the burned plots in the 2 week and 4 week post-burn samples (Figure 6.4B).

Nitrate levels clearly varied over time in response to burn treatment ($F = 8.821$, $p < 0.001$; Figure 6.4C). Two weeks after the burn, nitrate levels in the burned plots had doubled over pre-burn levels, whereas nitrate levels remained constant in the unburned plots. By the 6 week post-burn sampling date, nitrate levels in the burned plots had returned to the pre-burn levels. Burning had a significant but transient effect on prairie nitrogen dynamics.

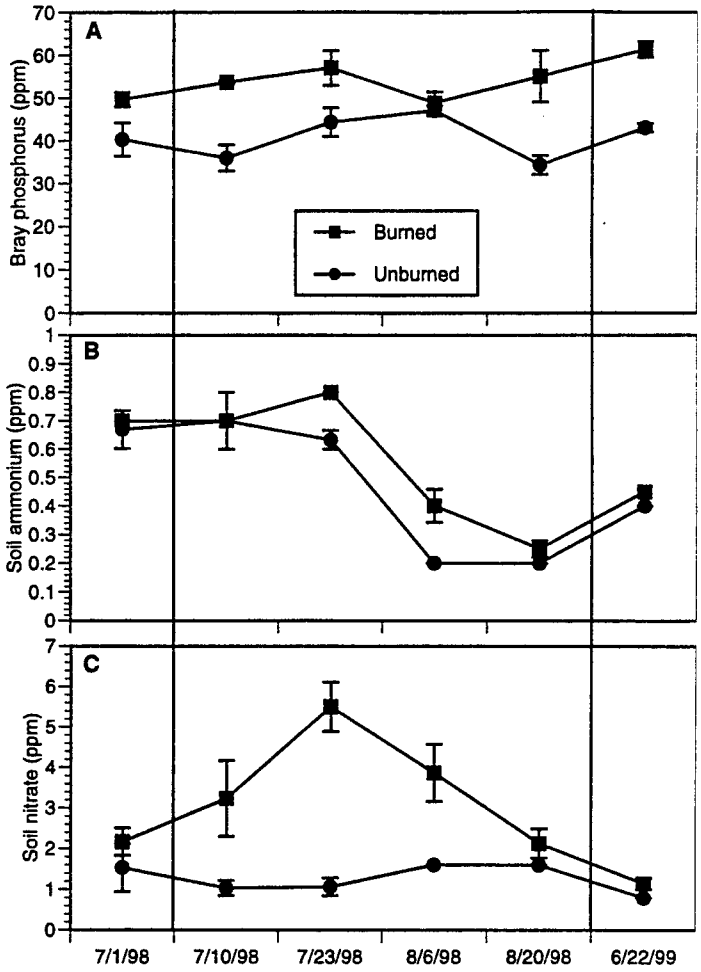

Figure 6.4. Soil nutrient content prior to burning (7/1/98) and following the July 9, 1998 burn at the JES restoration area in Cambridge, MN.

Mycorrhizal colonization tended to be higher in unburned plots. Averaged over all 1998 sampling dates, colonization did not differ significantly between treatments ($F = 2.608$, $p = 0.182$; Figure 6.5), but when considered individually, the July 10 (1 day post-burn) sampling date showed greater colonization in the burned plots. Similarly, vesicular colonization was significantly higher in burned than unburned plots at the 1 year post-burn sampling date ($F =$

9.271, p = 0.038). The probable explanation for this pattern is the lower levels of phosphorus in the unburned plots. As with the phosphorus data, this trend was extant before the onset of the experiment (p= 0.09). It is interesting, though, that colonization in the burned plots increased at the 2 week post-burn date such that it was actually slightly (but not significantly) higher than in the unburned plots. The fact that this corresponds to the peak in soil nitrate suggests a similar effect to that seen in Chapter 5: namely, that under N fertilization colonization increases, possibly through an alteration in the N/P balance. In upcoming prairie maintenance experiments, it would probably be worthwhile to continue to monitor mycorrhizal parameters.

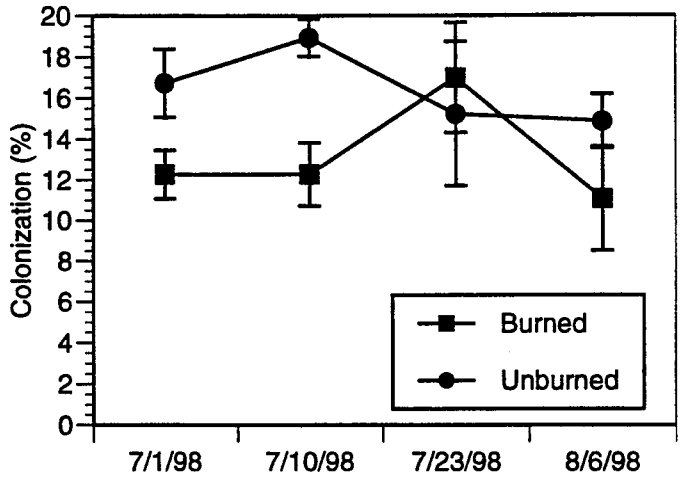

Figure 6.5. AM colonization prior to burning (7/1/98) and following the July 9, 1998 burn at the JES restoration area in Cambridge, MN.

Flowering in big bluestem was almost completely absent in the burned plots in 1998 (Figure 6.6), and was significantly lower than unburned plots (F = 37.4, p = 0.004). There was not sufficient time remaining in the season after the burn for big bluestem to successfully initiate flowering. However, by 1999, flowering in burned plots exceeded that in unburned plots, and statistically did not differ significantly between treatments (F = 1.352, p = 0.31). Consequently, it does not appear that a summer burn caused lasting harm to the reproductive capacity of big bluestem.

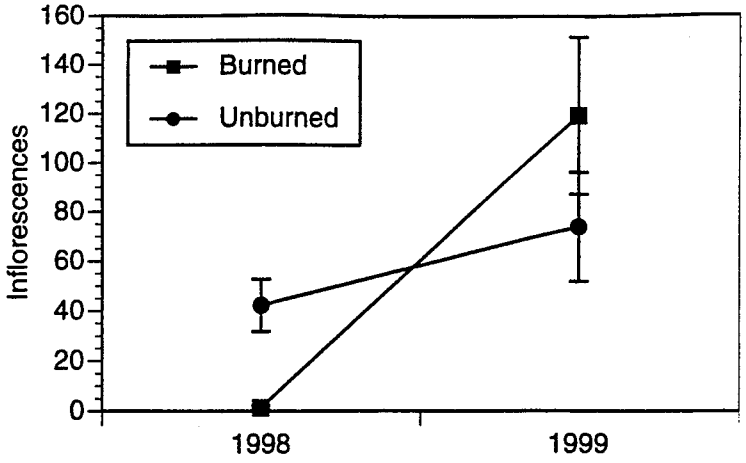

Figure 6.6. Number of big bluestem infloresences initiated in 1998 and 1999 following the July 9, 1998 burn at the JES restoration area in Cambridge, MN.

6.4 Conclusions

1) Burning/mowing studies at TH15 indicated an inconsistent effect of treatments on vegetation, which was likely due to differences between initial vegetation and soil characteristics between plots.
2) A small-scale burn experiment at JES showed that burning influenced soil characteristics for four weeks following the burn. There were some indications that mycorrhizal colonization might respond as well.
3) The early July burn at JES occurred too late to allow successful flowering of big bluestem that season. However, by 1999 big bluestem flowering in the burned plots didn't differ from unburned plots, indicating that the summer burn did not cause lasting harm to the reproductive capacity of big bluestem.
4) It is difficult to remove exotic species once they have become successfully established and are dominant at a site.

6.5 Recommendations

1) Future studies should a) have extensive pre-manipulation vegetation characterization, so that changes in vegetation can be accurately calculated and b) should try for more uniformity in vegetation composition or soil properties among experimental blocks.

2) Future burn/mow experiments should continue to monitor soil characteristics and mycorrhizal colonization.
3) Management energy might be more effectively spent in the early years of a restoration effort encouraging the growth of native plants. Once self-sustaining prairie communities were created, less energy input from Mn/DOT would be necessary. Maintenance should begin early after establishment, with no lag time between planting and management of newly restored areas.
4) Future studies should examine the timing and frequency of mowing and burning treatments to monitor the impact on exotic species. Certain management strategies might be more effective at reducing the cover of exotics.

Chapter 7. Long-term monitoring of mycorrhizal/plant characteristics of the JES restoration plots at Cambridge, MN

7.1 Overview

As described in Chapter 4, arbuscular mycorrhizal (AM) fungi can provide a number of benefits to their plant symbionts, including improved nutrient availability and increased drought tolerance [1]. Prairie ecosystems are typified by low nutrient availability, and many of the dominant plant community members are obligately mycorrhizal. Consequently, when prairie restoration efforts are undertaken, it is important to consider the availability of mycorrhizal propagules in the area to be restored. For example, prairie restoration along roadsides occurs on soil that has been highly disturbed, and often stockpiled for long periods of time. These conditions greatly reduce the inoculum potential of soil [6], and may make reestablishment of desirable plant species difficult. To improve the situation, one alternative is to introduce externally produced mycorrhizal inoculum to the target area. It has been hypothesized that such inoculation would promote growth of obligately mycorrhizal late-successional species over ruderal, early-successional species that are often non-mycorrhizal.

This hypothesis was substantiated by a field experiment by Smith et al. [5]. This study showed that mycorrhizal inoculation successfully increased mycorrhizal activity under field conditions: after 15 months, root colonization was significantly greater in inoculated plots than in uninoculated control plots. Moreover, the inoculated plots had greater percent cover of native planted species than the uninoculated control plots, supporting the conclusion that mycorrhizal inoculation can accelerate succession in a prairie restoration.

The purpose of this chapter is to report on the longer-term effects of mycorrhizal inoculation in these plots. Mycorrhizal colonization and vegetation cover have now been monitored at this site for five years. Given the expense and effort involved with the inoculation process, it is important to document whether long-term gain is achieved through mycorrhizal inoculation.

7.2 Materials and Methods

Twenty four 1 × 2 m plots were set up in June 1995 as described by Smith et al. [5]. Each plot received one of three treatments: native prairie seed + mycorrhizal inoculum, native prairie seed + sterile soil (control), or only native prairie seed (control) (hereafter referred to as the inoculated treatment, uninoculated soil control treatment and uninoculated control treatment, respectively). The inoculum was lab-produced via trap cultures from a local remnant prairie [5]. To minimize the effects of sampling on the vegetation, twelve out of the 24 plots were used to measure below-ground parameters and twelve were used to measure above-ground parameters, resulting in 4 replicate plots for each treatment.

7.2.1 *Below-ground parameters*

From 1997-1999, soil samples were taken annually during September. An additional set of samples was taken during July 1998. Soil cores were taken to a depth of 5 cm using a 2 cm diameter soil probe. In 1997, soil was sampled at 25 randomly chosen locations from each plot, half of which was used for root analysis, and half for mycorrhizal spore analysis. In 1998 and 1999, the number of soil cores for root and spore analysis was reduced to 10; however, in 1998 an additional five cores were taken per plot for use in soil nutrient analysis.

Roots were isolated over a 250 μm sieve, washed free of debris and preserved in 50% ethanol. Clearing and staining procedures followed methods modified from Kormanik and McGraw [40], Koske and Gemma [41], and Phillips and Hayman [53]. All roots obtained from the site were cleared overnight in 10% KOH, acidified for one hour in 1% HCl, stained overnight with 0.05% trypan blue and destained with acidic glycerol. A randomly selected subsample of the stained roots from each plot was mounted on microscope slides. Percentage AM colonization was determined using the magnified intersection method [21]. Roots were examined at 100 to 400× magnification. Approximately 200 intersections were examined for each plot. Total mycorrhizal colonization was calculated as the percentage of intersects that contained vesicles and/or arbuscules. Percent colonization was compared statistically among inoculation treatments using repeated-measures ANOVA at $\alpha = 0.05$.

Soil for spore analysis was dried in a convection oven, and spores were isolated from ~30g subsamples using sucrose density centrifugation with 38μm and 90μm sieves [modified from 18, 83]. Viable spores were then counted under a dissecting microscope at 10-63×

magnification, and spore density per gram dry soil was calculated. In 1998 and 1999, a 250μm sieve extraction layer was also examined, whereas this layer was excluded in 1997. Spore densities among treatments were compared statistically using repeated-measures ANOVA.

Soil samples collected in September 1998 were sent to the University of Minnesota Research and Analytical Laboratories, where NO_3-N and Bray-P were analyzed.

7.2.2 *Vegetative cover*

Percent cover measurements were also made annually in September, and in July of 1998. The point frame method was used, using a wooden frame that was constructed to fit over the one meter wide plots [76]. Ten flags were evenly spaced along this one meter distance on the frame. In 1997, the entire frame was placed ten times in each plot at 20 cm intervals yielding 100 points per plot. In 1998 and 1999 the number of sampling points per plot was reduced to 50. Each time an individual leaf, flower or stem touched the flag, its presence was recorded, even if it was from the same individual. Because multiple vegetation contacts could be made at a single point, percent cover could well exceed 100%. If the plant touching the flag was dead this was recorded next to the species code. If the plant could not be identified, it was either collected from outside the plot for future identification or recorded as an unknown. The 1998 and 1999 measurements were multiplied by two, to be comparable with previous measurements.
Vegetation cover was then compared statistically over time and among inoculation treatments using repeated-measures ANOVA.

7.3 Results and Discussion

7.3.1 *Mycorrhizal/soil parameters*

Overall, mycorrhizal colonization remained greater in the inoculated treatment than the uninoculated controls ($F = 8.395$, $p = 0.009$; Figure 7.1). The exception to this pattern was the summer 1998 data, where values were almost identical in all treatments. This suggests that treatment differences may only be apparent for part of the year.

Mycorrhizal colonization is apparently still increasing in all treatments. After five seasons growth, colonization is still lower at JES than at Shakopee after only two seasons (Chapter 5). This result is not surprising, given the much lower levels of initial colonization at the JES site (0.4%, [5]).

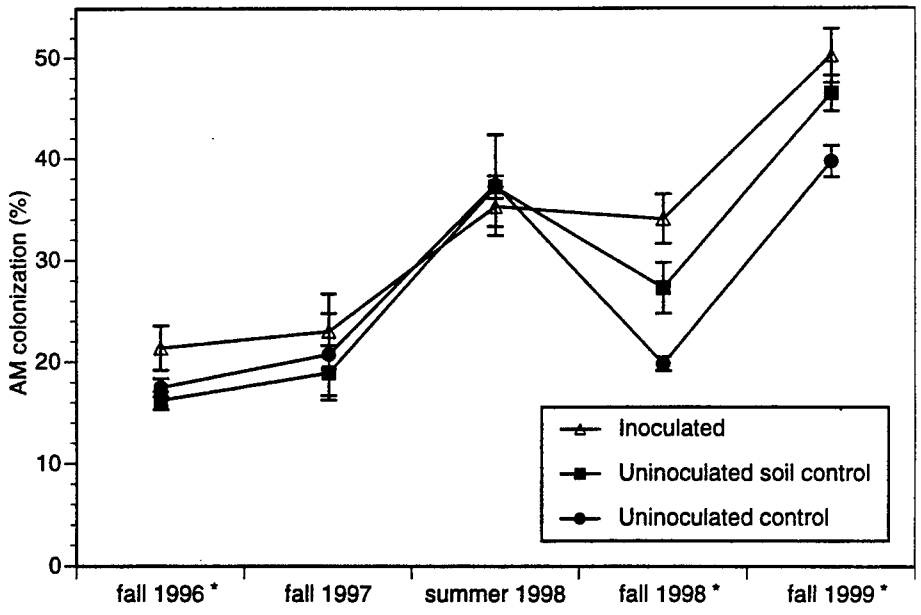

Figure 7.1. Mycorrhizal colonization of roots at the JES restoration plots near Cambridge MN. Stars represent sampling dates where significant differences were found among treatments at $\alpha = 0.05$.

Spore counts from soil collections in 1998 and 1999 were much higher than those of 1997 due to the difference in quantification technique (Table 7.1). In the 1998 and 1999 analyses, spores and sporocarps from the 250 μm sieve extraction layer were included in the total, whereas they were excluded in 1997. Consequently, the higher values (and greater

variance) obtained in later years most likely reflect a different measurement scheme, rather than a shift in field populations.

Although there was a trend towards lower spore densities in inoculated plots, this trend was not statistically significant ($F = 2.158$, $p = 0.172$). It should always be borne in mind, however, that spore density is highly variable, and not a particularly sensitive measurement of mycorrhizal activity.

Table 7.1. Mycorrhizal spore densities (spores/g dry soil) at the JES restoration plots.

	year		
Treatment	1997	1998	1999
Inoculated	1.2 ± 0.2	6.7 ± 1.3	12.2 ± 2.6
Uninoculated soil	1.5 ± 0.3	25.1 ± 7.4	14.0 ± 2.7
Uninoculated	2.1 ± 0.5	20.9 ± 7.1	18.1 ± 6.5

Soil nutrient analysis indicated that the plots did not differ in amount of nitrate or available phosphorus (nitrate $F = 0.582$, $p = 0.58$; phosphorus $F = 3.395$, $p = 0.08$; Table 7.2).

Table 7.2. Nutrient content of soil from the JES restoration plots, collected September 1998.

	Nutrient	
Treatment	NO_3 - N (%)	Bray - P (ppm)
Inoculated	0.73 ± 0.05	68.25 ± 3.47
Uninoculated soil	0.78 ± 0.03	61.25 ± 2.39
Uninoculated	0.76 ± 0.02	59.75 ± 0.63

7.3.2 *Vegetative cover*

When only fall samples are considered, native cover increased in all treatments over time (time effect $F = 7.025$, $p < 0.01$; Figure 7.2). The sole summer sample, in 1998, was substantially larger than any of the fall measurements. The lower fall values reflect the abscission of many grass leaves and the onset of senescence. Overall, percent native cover did

not differ significantly among the inoculation treatments ($F = 2.379$, $p = 0.148$). However, inoculated plots consistently had the highest native cover, significantly so for some individual sampling dates (fall 1996, summer 1998). Moreover, the lack of significance at later dates was not generally due to decreased magnitude of difference between treatment means, but rather due to increased variability within treatments.

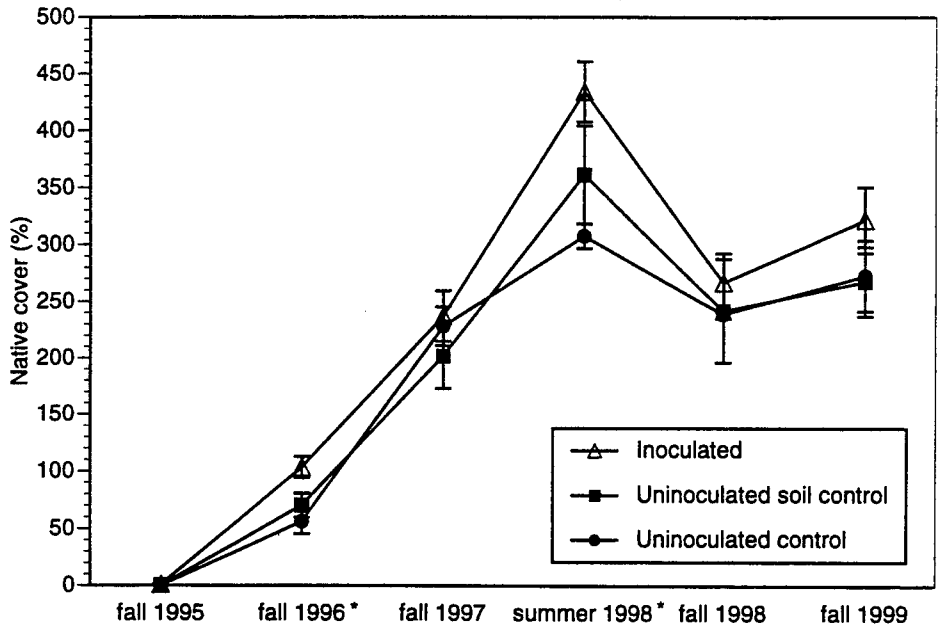

Figure 7.2. Percent cover of native species at the JES restoration plots near Cambridge, MN. Stars represent sampling dates where significant differences were found among treatments at $\alpha = 0.05$.

By fall 1997, native species accounted for most of the cover in all plots, whereas in 1996 only the inoculated plots had a majority of native species cover (Figure 7.3). By summer 1998, proportion native species (almost all warm season grass species) was greater than 90% in all treatments, and this was maintained in 1999. Regardless of mycorrhizal treatment, all plots were dominated by desirable native grass species.

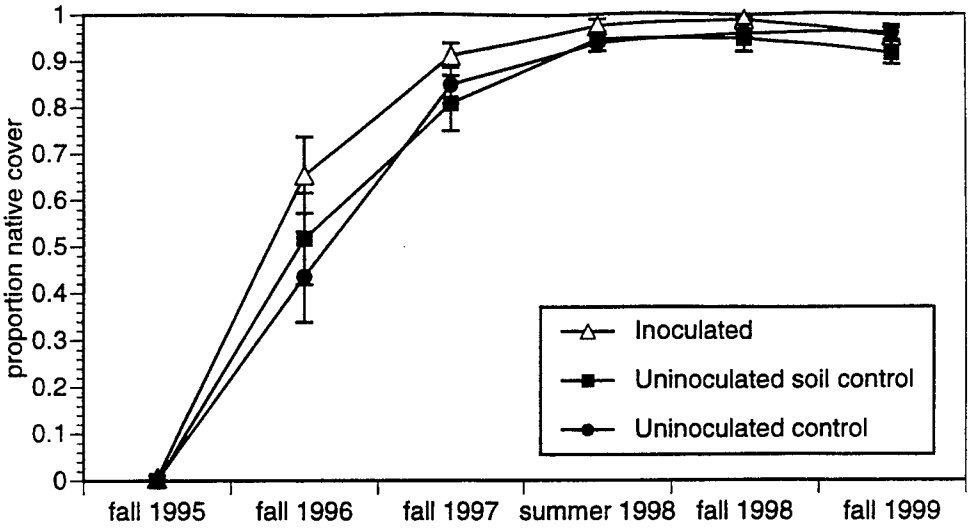

Figure 7.3. Proportion native cover out of total cover at the JES restoration plots near Cambridge, MN.

7.4 Conclusions

1) Mycorrhizal inoculation appears to have a lasting effect in terms of mycorrhizal colonization exhibited by plants.
2) There is some indication that native cover may still be greater in inoculated plots, but the effect is slight, and only statistically significant at some sampling points.
3) In terms of visual success of the restoration, mycorrhizal treatment appears not to have mattered: all plots are thickly covered by native grasses, regardless of mycorrhizal treatment. These results suggest that the benefits of inoculation occur primarily in first two years after establishment.
4) Mycorrhizal colonization and percent cover both appeared to vary seasonally. Summer sampling might be more sensitive in detecting differences in cover, but would miss differences in colonization.
5) Even if differences in cover were slight, there are other benefits that the plants in inoculated plots may have received, such as improved nutrient status or or improved drought stress tolerance. Percent cover was simply the only vegetation parameter that we measured.

Chapter 8. Monitoring mycorrhizal diversity of undisturbed Minnesota prairies for comparison to restoration sites

8.1 Overview

The purpose of this study is to quantify mycorrhizal diversity in undisturbed prairies, which will allow us to establish a baseline level of diversity against which to compare restoration siteScutellospora Bever et al. [84] have suggested that fungal diversity measurements directly reflect the intensity of sampling effort. To accurately assess mycorrhizal diversity at a site, multiple samples, sampling techniques, and culturing techniques must be used [84]. Given the intensive sampling needed to assess community diversity, this study has been restricted to prairie/savanna sites at Crosstown Prairie in Hennepin, Helen Allison Savanna Scenic Natural Area in Anoka County and Feder Prairie in Blue Earth County.

In addition to these studies at remnant prairies, trap cultures for mycorrhizal species propagation and identification were examined from Shakopee restoration and JES restoration upland and wetland sites. Spores from these sites have been characterized for comparison with mycorrhizal communities in restored versus native areas.

8.2 Materials and Methods

Soil from Crosstown Prairie, Feder Prairie and Helen Allison Savannah was collected and incorporated into trap cultures for mycorrhizal species propagation and identification (see Chapter 2). Spores were isolated from the resulting inocula via a sucrose centrifugation [18], segregated by type or species, and then used by type in a trap culture production with big bluestem as host. Pots were maintained in the greenhouse or the growth chamber, as described in Chapter 2.

Trap cultures for mycorrhizal species propagation and identification were also established from soil taken from Shakopee restoration and JES restoration upland and wetland sites. Dr. Hamdy Agwa, visiting professor from Egypt, characterized and identified the spores.

8.3 Results

Table 8.1 summarizes the fourteen mycorrhizal species identified from Crosstown Prairie. In addition to the species listed in Table 8.1, several Crosstown isolates were identified

to genera including an *Acaulospora spinosa* like isolate, four *Glomus* species and two *Scutellospora* species. One of the *Glomus* species had a characteristic hyaline thick wall and is hereafter referred to as *Glomus* species A.

Table 8.1. Species isolated and identified from Crosstown Prairie trap cultures from 1995-1997.

Arbuscular mycorrhizal species
Entrophospora infrequens (Hall) Ames & Schneider
Glomus constrictum Trappe
Glomus etunicatum Becker & Gerdemann
Glomus geosporum (Nicolson & Gerdemann) Walker
Glomus microcarpum Tulasne & Tulasne
Glomus mosseae (Nicolson & Gerdemann) Gerdemann & Trappe
Glomus occultum Walker
Gigaspora decipiens Hall & Abbott
Gigaspora gigantea (Nicolson & Gerdemann) Gerdemann & Trappe
Gigaspora margarita Becker & Hall
Scutellospora calospora (Nicolson & Gerdemann) Walker & Sanders
Scutellospora fulgida Koske & Walker
Scutellospora pellucida (Nicolson & Schenck) Walker & Sanders

A study of the mycorrhizal species identified from Helen Allison Savanna Scenic Natural Area soil is summarized in Table 8.2. Four species were identified. In addition to the species listed in Table 8.2, another isolate was identified as a *Glomus* species with a hyaline thick wall and appeared to be similar to *Glomus* species A identified from Crosstown Prairie.

Table 8 2 Species isolated and identified from trap cultures of Helen Allison Savanna Scenic Natural Area soil.

Arbuscular mycorrhizal species
Glomus etunicatum Becker & Gerdemann
Glomus occultum Walker
Scutellospora fulgida Koske & Walker
Scutellospora pellucida (Nicolson & Schenck) Walker & Sanders

In contrast to Crosstown Prairie, few AM species were found in trap cultures produced from Feder Prairie. Only *Glomus etunicatum* and *Glomus mosseae* were identified to species. The lack of mature spores in these trap cultures may be due to the high number of nematodes present in the trap cultures and in Feder Prairie soil. It is likely the nematodes used the AMF spores as a food source in both the field and trap cultures. Future AM studies on Feder Prairie soil should use culturing techniques that minimize nematode contamination of cultures. For example, the spores used in culturing could be isolated from field soil by sucrose centrifugation to help eliminated the nematode contaminants.

Table 8.3 summarizes the mycorrhizal species identified from the JES Upland restored prairie trap cultures. Soil samples were taken from inoculated and uninoculated plots in the summer of 1997, two years after the mycorrhizal inoculation at the JES Upland site (see Chapter 7). *Glomus mosseae, Glomus occultum* and *Scutellospora calospora* were found in both the inoculated and the uninoculated plots. Whereas, *Scutellospora fulgida* was isolated only from trap cultures made from the inoculated plot soil, and *Scutellospora pellucida* was found only in the trap cultures made from the uninoculated plot soil. This beginning study suggests that differences in the AM fungal community existed twenty-four months after the mycorrhizal inoculation at JES upland. It is also likely that the results reflect the limited number of samples studied.

Table 8.3 Species isolated and identified from JES Upland soil trap cultureScutellospora Soil samples were taken from inoculated and uninoculated plots in summer, 1997.

Arbuscular mycorrhizal species
Glomus mosseae (Nicolson & Gerdemann) Gerdemann & Trappe
Glomus occultum Walker
Scutellospora calospora (Nicolson & Gerdemann) Walker & Sanders
Scutellospora fulgida Koske & Walker
Scutellospora pellucida (Nicolson & Schenck) Walker & Sanders

Table 8.4 summarizes the mycorrhizal species identified from field soil isolated from JES Upland restored prairie. Soil samples were taken from inoculated and uninoculated plots in summer 1998, three years after the mycorrhizal inoculation at the JES Upland site (see Chapter 7). *Glomus mosseae, Scutellospora calospora* and *Scutellospora pellucida* were found in both the inoculated and the uninoculated plots. *Glomus constrictum* was found only in a control, but not in inoculated plots. On the other hand, *Glomus fasciculatum* was isolated only from inoculated plot. Again, this beginning study suggests that differences in the AM fungal community exited three years after the mycorrhizal inoculation at JES upland. The results likely reflect the limited number of samples studied.

Table 8.4 Species isolated from field soil from the JES Upland plots Soil samples were taken from uninoculated, uninoculated plus soil control, and inoculated plots in June, 1998.

Arbuscular mycorrhizal species
Glomus constrictum Trappe
Glomus mosseae (Nicolson & Gerdemann) Gerdemann & Trappe
Scutellospora calospora (Nicolson & Gerdemann) Walker & Sanders
Scutellospora pellucida (Nicolson & Schenck) Walker & Sanders

The Shakopee restored prairie trap cultures contained *Glomus etunicatum* Becker & Gerdemann, *Glomus occultum* Walker and a *Glomus species* with a hyaline thick wall.

Few studies have examined the mycorrhizal species found at roadside areas where standing water is present from spring to late fall as is the case at Country Club Site near Cambridge, MN. The species identified from Country Club site are summarized in Table 8.5. It is also likely that *Glomus occultum* Walker and *Glomus* species A are present at this site.

Table 8.5 Species isolated and identified from soil trap cultures from Country Club Site, Cambridge, MN in summer 1997.

Arbuscular mycorrhizal species
Entrophospora infrequens (Hall) Ames & Schneider
Glomus constrictum Trappe
Glomus microcarpum Tulasne & Tulasne

The species present at the JES Wetland restoration site are listed in Table 8.6. As is the case at the Country Club site, *Glomus occultum* Walker and *Glomus* species A appear to be present at this site. The JES wetland and Country Club sites are part of the same watershed: so similarities in AMF species would be expected.

Table 8.6 Species isolated and identified from JES Wetland soil trap cultures in summer 1997.

Arbuscular mycorrhizal species
Glomus etunicatum Becker & Gerdemann
Glomus geosporum (Nicolson & Gerdemann) Walker
Glomus mosseae (Nicolson & Gerdemann) Gerdemann & Trappe

8.4 Conclusions

Spore identification work was performed on trap cultures from JES upland and Helen Allison prairies, as a comparison to species found at Crosstown prairie. Of the 14 mycorrhizal species identified from Crosstown prairie, 4 have also been found at Helen Allison, and 5 have been identified in soil from JES upland. At Helen Allison, an additional three species that were not present at Crosstown have been identified, as well as a number of spore morphotypes which

require further work before positive identifications can be made. Similarly, at JES upland, two species that were not present at Crosstown have been identified, as well as 3-4 additional spore morphotypes. These results indicate that while some mycorrhizal species appear to be widespread, other species may be localized to specific geographic areas. Such AMF site specificity may be important to future restoration endeavors.

8.5 Recommendations

1) Further research should be conducted to identify additional AMF species present in Minnesota soils. True characterization of the communities will require long-term intensive work, involving mycorrhizal culturing and spore identification.
2) The relative abundance of different AMF species in given areas should be examined.

Chapter 9. Long-term monitoring of plant community composition at the JES restoration site at Cambridge, MN

9.1 Overview

In 1994 an area north of Cambridge, Minnesota was severely disturbed when the Minnesota Department of Transportation (Mn/DOT) constructed a new roadway (MN state highway 65). After construction was completed, the area was modified so that both wetland (JES pond) and drier upland areas were created. Both fill and the original topsoil were used, and a cover crop of annuals and short-lived perennials was planted at that time to control erosion. In 1995, both upland and wetland areas were seeded with a native prairie mix, and many native wetland species were planted at plots south and east of JES pond by Dr. David Biesboer from the U of MN Plant Biology Department and Robert Jacobson, supervisor of the Mn/DOT turf establishment and erosion control unit.

The purpose of this chapter is to document the vegetational composition and long-term success of the restoration efforts at the JES site. Three approaches were taken. First, a walk through inventory of plant species composition was conducted, for comparison of extant species with species seeded and planted at the site. Second, permanent vegetation plots were set up, and species composition in 1996 versus 1999 was compared. Third, the composition of the seed bank was examined and compared to extant vegetation. From these studies, it became clear that *Phalaris arundinacea*, reed canary grass, is a problem at the site. Consequently a number of undergraduate students have undertaken small scale projects to try to understand the biology of this invasive weed.

9.2 Overall species list

9.2.1 *Materials and methods*

The JES species list was compiled from three major sources: surveys of the permanent plots conducted in summer 1999 (see section 9.3), surveys of the seed bank plots conducted in 1998 (see section 9.4), and from a walk through survey conducted 9/98. No effort was made to quantify species abundance in any of the surveys, nor was special effort made to seek out planted or seeded species. Once compiled, the species list was compared to the lists of species seeded and/or planted at the site.

9.2.3 *Results and discussion*

While not an exhaustive list, more than 100 species have been identified at JES. Eighteen seeded species were observed. Large portions of the upland areas are dominated by seeded warm season grasses, particularly big bluestem (*Andropogon gerardii*), switchgrass (*Panicum virgatum*), and Indian grass (*Sorghastrum nutans*). Seeded forbs such as black-eyed susan (*Rudbeckia hirta*), common ox-eye (*Heliopsis helianthoides*), and wild bergamot (*Monarda fistulosa*) are also quite common. A few seeded forb species were not observed, including partridge pea (*Chamaecrista fasiculata*), showy penstemon, (*Penstemon grandiflorum*), and butterfly milkweed (*Asclepias tuberosa*).

At least twenty-one of the species planted in 1995 were still present at the site in 1998. Additionally, gentian and sunflower were both found at the site, but not identified to species. In all probability, these represent two more of the planted species, prairie bottle gentian (*Gentiana andrewsii* and giant sunflower (*Helianthus giganteus*). Only two of the planted species were not observed: culver's root (*Veronicastrum virginianum*) and fringed loosestrife (*Lysimachia ciliata*). However, absence from this survey does not equate with absence from the site, and it is quite possible that these species are actually present. In general, most of the planted species are not widespread, and many probably have not spread far beyond the original plantings, but most seem well established. The lobelias, in particular, appear to be flourishing and spreading.

Despite the relative success of the seeded and planted species, there are a number of vegetational "problems" at the site. Reed canary grass (*Phalaris arundinacea*) has formed nearly monotypic stands along some portions of the western shore of the pond and continues to spread, although another strong competitor, stinging nettle (*Urtica dioica*), seems to be holding its own. Birdsfoot trefoil is a non-indigenous species that has high prevalence at the upland portions of the site, and appears to be increasing throughout. To the east of JES pond, nearest state Highway 65, sweet clover (*Melilotus alba* and *Melilotus officianalis*), has become widespread, and may eventually displace the native grasses in that portion of the site. The single individual of purple loosestrife (*Lythrum salicaria*) found at the site was uprooted and removed. It should also be noted that erosion along the steep slopes found in many parts of the site has led to a fair amount of bare ground, uncovered by any vegetation.

Table 9.1 Plant species identified at the JES restoration site, Cambridge, MN.

Scientific Name	Common name	Seeded species	Planted species
Acer sp.	maple		
Achillea millefolium	common yarrow		
Alnus incana	speckled alder		
Ambrosia artemisiifolia	common ragweed		
Ambrosia trifida	giant ragweed		
Anaphalis margaritaea	pearly everlasting		
Andropogon gerardii	big bluestem	X	
Anemone canadensis	Canadian anemone		X
Antennaria plantaginifolia	pussytoes		
Artemesia ludoviciana	white sage		
Asclepias incarnata	swamp milkweed		X
Asclepias syriaca	common milkweed		
Aster novae-angliae	New England aster	X	X
Aster sp.	aster		
Aureolaria sp.	false foxglove		
Berteroa incana	hoary alyssum		
Bidens cernua	beggars tick		
Bouteloua curtipendula	side-oats grama	X	
Bouteloua gracilis	blue grama	X	
Bromus ciliatus	fringed brome		
Calamagrostis canadensis	bluejoint grass		X
Caltha palustris	marsh marigold		X
Carex comosa	bottlebrush sedge		X
Carex hystericina	sedge		
Carex retrosa	sedge		
Carex scoparia	sedge		
Carex stipata	sedge		
Carex vulpenoidea	fox sedge		
Chelone glabra	white turtlehead		X
Chrysanthemum leucanthemum	ox-eye daisy		
Cicuta bulbifera	water hemlock		
Cirsium arvense	Canada thistle		
Cornus sericea	red osier dogwood		
Dalea candidum	white prairie clover	X	

Table 9.1, continued

Scientific Name	Common name	Seeded species	Planted species
Dalea purpureum	purple prairie clover	X	
Digitaria ischaemum	smooth crabgrass		
Echinochloa sp.	Barnyard grass		
Elocharis sp	spike rush		
Elymus canadensis	Canada wild rye	X	
Epilobium ciliatum	American willow herb		
Equisitum sp.	horsetail		X
Erigeron sp.	fleabane		
Eupatorium maculatum	spotted joe-pye weed		X
Eupatorium perfoliatum	boneset		X
Fragraria sp.	strawberry		
Gentiana sp.	blue gentian		?
Geranium maculatum	wild geranium		
Glyceria grandis	American mannagrass		X
Helianthus sp.	sunflower		?
Heliopsis helianthoides	common ox-eye	X	
Impatiens capersis	jewelweed		
Iris versicolor	northern blue flag iris		X
Juncus effusus	soft rush		
Juncus tenuis	path-rush		
Juncus sp 3	rush		
Leerzia orzoides	rice cutgrass		
Lemna sp.	duckweed		
Lespedeza sp.	bush clover		
Liatrus sp.	blazing star	X	X
Lobelia inflata	Indian tobacco		
Lobelia siphilitica	great blue lobelia		X
Lotus corniculatus	birdsfoot trefoil		
Lythrum salicaria	purple loosestrife		
Melilotus alba	white sweet clover		
Melilotus officianalis	yellow sweet clover		
Mimulus ringens	monkeyflower		X
Monarda fistulosa	wild bergamot	X	
Muhlenbergia mexicana	wirestem muhly		

Table 9.1, continued

Scientific Name	Common name	Seeded species	Planted species
Oenothera biennis	common evening primrose		
Panicum virgatum	switchgrass	X	
Penthorum sedoides	ditch stonecrop		
Phalaris arundinacea	reed canary grass		
Phleum pratense	timothy		
Physostegia virgiana	obedience		
Plantago sp.	plantain		
Poa pratensis	Kentucky bluegrass		
Polygonaceae sp.			
Populus deltoides	eastern cottonwood		
Potentilla simplex	oldfield cinquefoil		
Prunella vulgaris	self-heal		
Pycnanthemum virginianum	mountain mint		X
Ratibida pinnata	gray-headed coneflower	X	
Ribes sp.	gooseberry		
Rubes sp.	raspberry		
Rudbeckia hirta	black-eyed susan	X	
Rudbeckia laciniata	cutleaf sunflower		
Salix sp 1	willow		
Salix sp. 2	willow		
Schizachrium scoparium	little bluestem	X	
Scirpus atrovirens	black bulrush		X
Scirpus cyperinus	wool grass rush		
Scirpus sp.	bulrush		
Scirpus validus	softstem bulrush		X
Setaria faberi	giant foxtail		
Setaria glauca	white foxtail		
Silene latifolia	white campion		
Solidago sp. 1	goldenrod		
Solidago sp. 2	goldenrod		
Sorghastrum nutans	Indian grass	X	
Spartina pectinata	prairie cord-grass		X
Sporobolis crytandrus	sand dropseed	X	
Stelaria sp.	chickweed		

Table 9.1, continued

Scientific Name	Common name	Seeded species	Planted species
Taraxacum officinale	dandylion		
Thalictrum dasycarpum	purple meadow rue		X
Tragopogon sp.	goat's beard		
Trifolium pratense	red clover		
Trifolium repens	white clover		
Typha spp.	cattail		
Urtica dioica	stinging nettle		
Verbascum thapsus	common mullein		
Verbena hastata	blue vervain	X	
Verbena stricta	hoary vervain	X	
Zizia aurea	golden alexander		X

9.3 Permanent vegetation plots

9.3.1 *Materials and methods*

Five randomly located 1m x 2m plots were set up along the north-western shore of JES pond; 3 plots were located at the water's edge in late June, 1996 when the first sampling was conducted, and the other 2 were located approximately 6 m from the June high water point on the north shore. Monthly surveys of plant composition in these plots were made in 1996, wherein percent cover of all species was visually estimated. Specimens of species that could not be identified in the field were brought back to the lab for identification. Four of the five plots were located again in 1999, and vegetation was surveyed in August. One plot could not be found due to the change in water level. For the four discovered plots, species number and diversity in August 1999 were compared to species number and diversity in August 1996.

9.3.2 *Results and discussion*

The vegetation composition of the permanent plots has shifted considerably over three years. In three of the four plots, the percent cover of reed canary grass, *Phalaris arundinacea*, has increased; average coverage by reed canary grass in the four plots was 27.5% in 1996, versus 45% in 1999. Overall, the average number of plant species present per plot is higher in 1999 than 1996 (11.75 versus 9). However, average species diversity (which takes into account both

number and equitability of species) was lower in 1999 than 1996. This indicates that on average, the plots currently exhibit greater dominance by one or a few species than they did in 1996.

9.4 Seed bank profile

The goal of this project was to provide a baseline of information concerning the seed bank developing in a Mn/DOT roadside restoration area. Seed banks are a potential source of seed for revegetation of areas that have been disturbed. The seed numbers and their depth within the sediment can provide an indication of the potential for plant reestablishment on disturbed areas [85]. The complete vegetation profile of any restoration site should include a review of the seed bank as it may represent both current and future vegetation.

9.4.1 *Materials and methods*

Study methods

The reconstructed wetland at the Cambridge, Minnesota, site (JES) was divided into 2 m x 2 m plots. Eighteen plots were randomly selected for study. Vegetative cover was estimated in early October, 1998, for each plot using a Bran-Blanquet scale: r = 1 individual with insignificant cover, + = few individuals with insignificant cover, 1 = many at 1-5%, 2 = 5-25%, 3 = 25-50%, 4 = 50-75%, 5 = 75-100% cover [86].

Each of the eighteen plots was marked in the NE corner with a five-foot metal rod. Seven randomly located soil cores 10 cm in depth and 5 cm in diameter were removed from each plot in late March, 1998. Soil cores were collected after seed dispersal, dormancy, and stratification had occurred. Soil cores were kept on ice while transported to a 4°C cold-room, where they were kept for two weeks.

For each plot the top 5 cm of the seven cores were combined and thoroughly mixed; roots and debris were removed. Half of this seed bank soil was divided into three equal subsamples, each placed in a different holder. Holders were 19 cm square by 5.5 cm high (Perma-Nest, Growers Supply Co., Ann Arbor, MI) with five drainage holes. Holders were lined with polyester fiber, filled with 1000 cm^3 of washed and heat sterilized sand (Minispheres, Unimin Corp.), and then layered with the seed bank soil. A total of 57 holders (18 plots * 3 subsamples, + 3 sterile controls) were placed in containers and the surrounding water level was maintained at ~2 cm below the sand surface. Greenhouse temperatures averaged 14 °C at night and 25 °C

during the day during April, May, and June. High intensity lighting was used 12-15 hours daily. From July through September high intensity lights were not used and the temperature range was closer to outdoor fluctuations. No germination was recorded in the three control holders containing only 1000 cm^3 of sterile sand.

The seedlings were checked every other day for six months until taken down at the end of September, at which time new seedling germination had stopped in all holders. Records were kept of identifications, removals, and deaths. The holders were randomly rotated every week and containers refreshed with tap water as needed three to four times weekly. Seedlings which could not be identified were grown out for later identification and confirmation at the University of Minnesota Herbarium. Plant nomenclature follows Gleason and Cronquist [87].

Data analysis

Seed density is expressed as the number of seeds per square meter, to a soil depth of 5 cm. Similarity between the vegetation and the seed bank was calculated using Sørenson coefficient of community [88]. The formula was $CC= 2M_C/ (M_A + M_B)$ (M_C was the total of all species common to both seed bank and vegetation and $M_{A,B}$ was the sum of all species in seed bank and vegetation). Weighted average analysis of wetland seed bank species in each plot was done using categories taken from Wetland Plants of the United States of America 1986 [89]. We gave each of Reed's wetland categories a value (5 = obligate (>99% frequency in wetlands), 4 = facultative wetland (67%-99% frequency in wetlands), 3 = facultative (34%-66% frequency in wetlands), 2 = facultative upland (1%-33% frequency in wetlands), and 1 = non-wetland (1% frequency in wetlands)) and used seed numbers for the weighting. The formula is $W_j = I_{ij}E_{ij}/ I_{ij}$ where W_j = weighted average for stand j, I_{ij} = importance value for species i in stand j (seed number), and E_{ij} = ecological index for species i (wetland category number) [90].

9.4.2 *Results and discussion*

A total of 2,125 seedlings were counted in this seed bank study, bringing seed density to 17,209 seeds/m^2 (Table 9.2). Sixty different species were found (Table 9.3). The most significant contributor to total seed bank numbers was *Juncus tenuis,* accounting for 28.0% of the total. It is not uncommon for one species to dominate freshwater wetland seed banks and for the dominant species to be a monocot and a graminoid from Poaceae, Cyperaceae, or Juncaceae

[91]. The ten seedlings with the highest seed density accounted for 80.0% of the seeds counted. Five of these species were obligate wetland plants, three were facultative wetland plants, and two were facultative upland plants. A weighted average of seed numbers and wetland category for all seedlings classified the seed bank between facultative and facultative wetland at 3.39.

Table 9.2. Mean seed density (± SE) profile of all species found in the JES seed bank in March 1998 (n = 18).

Seed bank measurement	
Total density/m^2	17,209 ± 5,410
Density of perennials/m^2	13,654 ± 5,305
Species/plot	15.2 ± 1.42
Perennial species/plot	8.44 ± .994
Wetland index*	3.39± .137
Total species	60

* See materials and methods section

Table 9.3. Total seedling numbers and relative density of the top ten species in the JES seed bank in March 1998.

	Total seedling numbers	Relative density	Life history
Juncus effusus L.	594	28.0%	Perennial
Juncus tenuis Willd.	245	11.5%	Perennial
Carex L. sp.	197	9.3%	Perennial
Penthorum sedoides L.	188	8.8%	Perennial
Scirpus L. sp.	133	6.3%	Perennial
Cerastium nutans Raf.	122	5.7%	Annual
Plantago major L.	76	3.6%	Perennial
Potentilla norvegica L.	52	2.4%	Annual
Typha L. sp.	47	2.2%	Perennial
Verbascum thapsus L.	45	2.1%	Annual
Total seed bank seedling numbers	2,125	100.0%	

Of the 55 species with life span identified, 23 were annuals and 32 were perennials; 21 different families were represented (Table 9.4). Of the 60 species identified as herbaceous or graminoid plants, 24 were graminoids and 36 were herbaceous. Of the 51 species identified as

indigenous or non-indigenous, 35 were indigenous and 16 were non-indigenous. The majority of seed bank species were not part of the original restoration seeding. Only nine of the 60 species found in the seed bank were included in the original seeding efforts at the Cambridge site.

Table 9.4. The number of seed bank species per family found at JES in March 1998.

Family	species number	Family	species number
Poaceae	16	Campanulaceae	1
Asteraceae	6	Chenopodiaceae	1
Cyperaceae	5	Lemnaceae	1
Scrophulariaceae	4	Oxalidaceae	1
Caryophyllaceae	3	Plantaginaceae	1
Juncaceae	3	Rosaceae	1
Onagraceae	3	Saxifragaceae	1
Brassicaceae	2	Typhaceae	1
Fabaceae	2	Urticaceae	1
Alismataceae	1	Verbenaceae	1
Apiaceae	1		
Total			57

The species richness of vegetation was lower than the seed bank. Sørenson coefficient of community was 0.32 between all vegetation and all seed bank species indicating low similarity. The lack of similarity between vegetation and seed bank in wetlands is frequently reported [92, 93, 94, 95]. An analysis of 14 restored freshwater wetland areas found means of similarity between vegetation and seed bank ranging from 0.15 to 0.25 over a three-year period using the Jaccard index [95]. Jaccard measurements for our site are 0.19 between all seed bank and all vegetation.

Seed density at JES falls within the broad range of 11 to 36,639 seeds/m^2 reported for natural North American freshwater wetland seed banks [91], but is higher than numbers reported for restored freshwater wetland which are near 10,000 seeds/m^2 [96, 95]. The species richness of 60 is also high for restored wetlands. The high seed density and species richness in the seed bank at JES may be partially explained by the nearness of JES to a large and well-established shrub swamp. Increased complexity of surrounding vegetation may increase seed bank diversity

[97,98]. The low number of species from the original seeding is another indication that new seed from the surrounding area is important to early seed bank development at this site.

Phalaris arundinacea has become a significant presence at this wetland in the first three years of vegetation development (estimated at 15% of total cover in spring 1998). In contrast to the vegetation, the seed bank contained less than 1% *P. arundinacea* seedlings. The low seedling count of *P. arundinacea* may simply reflect the young age of this restoration. In a study of prairie pothole wetlands, *P. arundinacea* had a higher density and was more frequently found in natural than in three-year-old wetland seed bank [96].

9.5 Studies of reed canary grass biology

Reed canary grass (*Phalaris arundinacea*) is a vigorous cool season perennial grass that can tolerate a wide range of environmental conditions, but is particularly prone to forming dense swards in areas subject to disturbance [99]. While these characteristics make reed canary grass a useful grass for forage and other agricultural applications, they also can make it a tremendous problem in restoration areas, making establishment of more desirable species difficult.

In an effort to understand more about the biology of this species, three undergraduate interns have undertaken projects focusing on reed canary grass. In particular, they have examined the mycorrhizal status of this species in relation to native, more desirable, species found at JES to find out whether arbuscular mycorrhizae might play a role in the competitive ability of reed canary grass.

9.5.1 *Materials and methods*

In June 1998, Julie Rose, a summer intern with the NSF Aquatic Environmental Sciences Program, collected three randomly chosen reed canary grass plants from a transect along the north side of JES pond, and three randomly chosen bluejoint grass (*Calamagrostis canadensis*) plants from a transect from a less disturbed wetland area WSW of JES pond. In each area she collected soil samples for comparison of nutrient content. All samples were placed on ice and transported back to the laboratory. Roots from each plant were carefully isolated from the root mass, cleared with 10% KOH, stained with 0.05% trypan blue in lactoglycerol [40] and mounted on slides to determine percentage colonization using the magnified intercept method [21]. Portions of the soil samples were compiled for each plant species, and sent to the University of

Minnesota Research and Analytical Laboratories for analysis of soil moisture, nitrate, ammonium, Bray extractable phosphorus, carbon, organic material, and pH.

In August 1998, this study was continued by Hien To, as part of the Undergraduate Research Opportunities Program (UROP). Five additional samples of each plant were collected, and processed in a like manner. Soil samples were analyzed individually for moisture, nitrate, ammonium, and Bray-P, rather than being compiled. Each student compared percent colonization and spore density between species using two-sampled t-tests.

In June 1999, Sarah Goetz, another NSF Aquatic Environmental Sciences Program intern, compared the mycorrhizal status of reed canary grass growing in wetland and upland habitats to that of adjacent native species, thus controlling environmental variation to some degree. As no other species was found in as broad a range of habitats as reed canary grass, each upland reed canary grass plant was paired with a nearby (<1m) little bluestem *(Schyzachrium scoparium)*, and each wetland reed canary grass plant was paired with a nearby cattail (*Typha glauca*). A total of 5 upland and 6 wetland pairs of plants were extracted, and root material was processed and examined for percent colonization as described above. Additionally, two soil cores were taken near each pair of plants and bulked, for a total of 11 soil samples. The samples were sent to the University of Minnesota Research and Analytical Laboratories and individually analyzed for Bray-P and % moisture content. An additional set of 6 upland and 8 wetland pairs of plants, + soil samples, were collected in August 1999, and analyzed in a like manner.

9.4.2 Results and discussion

In the June 1998 samples, AM colonization was significantly greater in bluejoint grass than in reed canary grass (Figure 9.1). In contrast, there were no significant differences in colonization between the plant species in August 1998, and the observed trend was in the opposite direction, towards greater colonization in reed canary grass than bluejoint grass. Moreover, colonization values for reed canary grass show little congruity between sampling dates, with approximately 10× greater colonization observed in August than in June. A likely explanation for this disparity is that the root samples of the two students differed in their coarseness. Julie Rose's June roots were, on average, much coarser than Hien To's August samples. AM fungal colonization is typically highest in fine branch roots, by viewing

predominantly coarse roots, Julie Rose likely arrived at a much lower estimate of overall colonization.

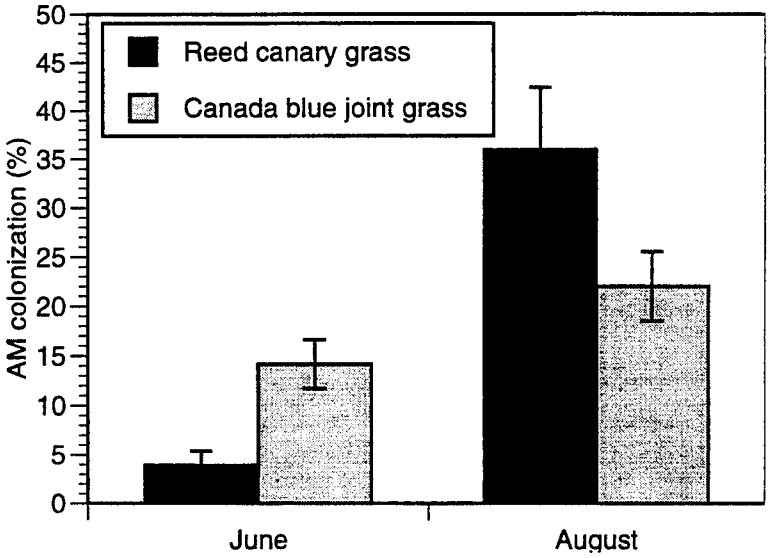

Figure 9.1. Mean (± 1 S.E.) AM colonization of reed canary grass and Canada bluejoint grass collected from JES wetland in 1998.

In terms of soil parameters, there were many differences between the more-disturbed area where reed canary grass was sampled and the less-disturbed area where bluejoint grass was sampled (table 9.5). Soil in the bluejoint grass area was typical of wetland soils: high ammonium, moisture content, and organic material, low pH. Soil in the more disturbed area where reed canary grass was sampled was not very wetland-like, and bore more resemblance to upland soil than wetland soil (see chapters 6 & 7 for upland soil measurements at this site). The seasonal nitrogen dynamics also appeared to differ between sites: from June to August ammonium decreased in the more disturbed area, but increased in the less disturbed area.

Table 9.5. Soil characteristics of areas where reed canary grass (more disturbed) vs. bluejoint (less disturbed) grow at the JES restoration site, Cambridge, MN.

	NO_3 (ppm)	NH_4 (ppm)	percent moisture	% organic material	percent carbon	pH	Bray-P (ppm)
Reed canary grass							
June 1998	1.1[a]	1.2	24.9	1.9	1.0	6.7	9
August 1998	0.8 ± 0.02	0.5 ± 0.06	26 ± 2	1.9	1.0	7.0	13.8 ± 4.0
Bluejoint grass							
June 1998	0.9	6.9	69	28.7	14.2	5.5	10
August 1998	0.9 ± .08	12.7 ± 2.6	69 ± 8	19.1	10.6	5.3	12.8 ± 6.1

[a] Mean ± 1 SE. Values without standard errors were compiled into a single sample prior to analysis.

In 1999, when site effects and sampling biases were controlled for, arbuscular colonization of reed canary grass was greater than colonization in adjacent little bluestem in the upland and much greater than in adjacent cattails in the wetland (Figure 9.2). Colonization of all species appeared to decline slightly from the June to August sampling dates. Reed canary grass colonization was significantly negatively correlated to soil moisture (Figure 9.3), such that colonization was significantly lower in the wetland than the upland. It is noteworthy, however, that some of the reed canary grass samples from the wettest sites had substantial colonization.

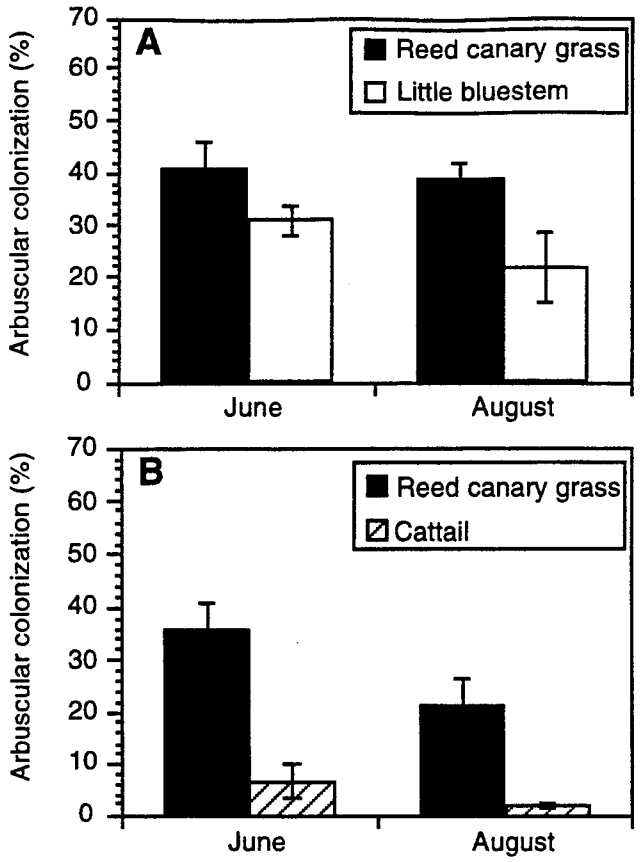

Figure 9.2. Mean (± 1 S.E.) AM colonization of reed canary grass, little bluestem (A) and cattail collected from JES wetland in 1999.

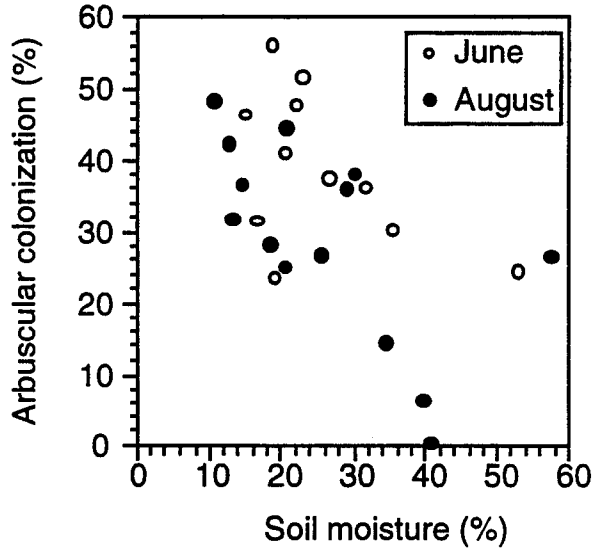

Figure 9.3. AM colonization as a function of soil moisture at JES wetland, 1999.

9.6 Conclusions

1) The JES restoration site has a diversity of plant species in both the upland and wetland areas, many of which are indigenous desirable species.
2) Most of the planted and/or seeded species remain present at the site, and some are flourishing.
3) Seed density on the JES site is higher than that found at most wetland restorations.
4) The seedbank at JES is diverse, contains a high proportion of indigenous seed species, but is fairly dissimilar to the vegetative community at the site.
5) Non-indigenous invasive species such as reed canary grass and birdsfoot trefoil are increasing in prevalence at the site, and threaten the overall diversity of the site.
6) It appears unlikely that the seedbank was the source of the non-indigenous problem species at the JES site, as there was no evidence of a persistent presence of reed canary grass or birdsfoot trefoil in the seedbank.

9.7 Recommendations

1) Experiments designed to determine the influence of reed canary grass litter on seedling recruitment and species composition early in the restoration process at reconstructed wetlands should be considered.
2) To determine likely sources of non-indigenous invasive species, factors such as geographic distance from and hydrological connection to propagule sources for undesirable species should be examined.
3) Control measures, such as appropriately timed burns, may be used to control non-indigenous invasive species.
4) The presence of weedy seeds in the seed mix and/or mulch used at the site should be investigated, and if found, eliminated.

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