ABSTRACT

ARBUSCULAR MYCORRHIZAL FUNGAL COMMUNITY STRUCTURE
IN A MANIPULATED PRAIRIE AND THE IMPACT ON PLANT COMPETITION

By Jeremiah A. Henning

The North American tallgrass prairie once stood the continent's largest ecosystem. The magnitude of this grassland has been significantly reduced via sustained agriculture. Contemporary ecologists are striving to restore these lost habitats with limited success. Within their roots, many prairie plants harbor symbiotic fungi called arbuscular mycorrhizal fungi. The fungi assist the plants in nutrient acquisition in return for carbohydrates as a food source. Mycorrhizal fungal sporulation was examined in an 8.1-ha reconstructed prairie in Eau Claire County, Wisconsin. In the fall of 2003, the site was planted with differing combinations of native prairie species. It was hypothesized that diverse plant seeding mixtures would promote mycorrhizal fungal diversity. To further test the interaction between plant and fungal communities, each plot was subdivided and treated with the fungicide, chlorothalonil, to suppress mycorrhizal fungi or ammonium nitrate fertilizer, to mimic common agricultural practices. Fungal sporulation within the subplots was impacted by both fungicide and fertilizer treatments. Suppression of mycorrhizal fungi also caused changes in the relative abundance of grasses in the plant community.

Based on this observation, two greenhouse experiments were conducted to address the role of mycorrhizal fungi in the competitive ability of two native prairie grasses (*Sorghastrum nutans* and *Andropogon gerardii*). The first experiment assessed plant competition by growing the two plant species in a pair-wise combination while suppressing mycorrhizal fungi with fungicide. In the second experiment, plants were inoculated with known mycorrhizal fungal communities in sterilized soil. Mycorrhizal fungi impacted the competitive ability of the grasses as well as the overall root architecture of both species.

The results reinforce the importance of mycorrhizal fungi in the structuring, stability and productivity of plant communities. Successful restoration of lost prairie habitats will have to account for its underground fungal symbionts.

ARBUSCULAR MYCORRHIZAL FUNGAL COMMUNITY STRUCTURE
IN A MANIPULATED PRAIRE AND THE IMPACT ON PLANT COMPETITION

by

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CHAPTER I

INTRODUCTION

Prairie History -- Four hundred years ago, the North American prairie covered millions

of hectares from Indiana, westward to the Rocky Mountains, and from Texas northward

to Southern Canada (Risser et al 1981). The immense area made up the continent's

largest ecosystem (Risser et al 1981). These grasslands developed about 135 Mya, soon

after the up-lift of the Rocky Mountains at the end of the Mesozoic period (Kline 1997,

Risser et al 1981). This mountain formation caused a shift in the precipitation patterns in

central North America, leaving a dry environment that was conducive to the grasses that

dominated the landscape (Kline 1997). It was also this dry environment that fueled

frequent natural wildfires which maintained the prairie boundary and impeded the

encroachment of the oak forests (Kline 1997, Steinauer and Collins 1996).

These grasslands were a highly productive ecosystem and an area of high

biological diversity (Kline 1997). The typical prairie plant community is a diverse place

composed of a few hundred species per hectare; with commonly represented families

being the grasses (Poaceae), composites (Asteraceae), and legumes (Fabaceae)

(Kline 1997). The prairie ecosystem was structured by the frequent disturbances that

spread across the open landscape such as frequent fires, droughts, and animals burrowing

and grazing, which removed vegetation from patches (Steinauer and Collins 1996). The

disturbances led to the heterogeneity of the landscape, which allowed the diversity of

flora. These grasslands flourished in this environment until the time of European settlement when massive anthropogenic disturbance commenced.

Europeans were amazed by the expanse of this grassland and were unable to describe such a place. The French called this place *pre'rie* (meadow), from which we derive the word "prairie" (Kline 1997, Risser et al 1981). Settlers to this area capitalized on the high productivity of these grasslands and began cultivating these fertile soils, transforming it into what would become America's bread basket. The natural disturbance regimes that acted upon the community were significantly altered, and many novel disturbances were introduced (Steinauer and Collins 1996). Settlers began plowing and mowing the plant communities, as well as eventually applying herbicides and fertilizers to the nutrient limited soils. Grasslands were quickly transformed to monocultural stands of mostly corn and wheat. Settlers also introduced many exotic plant species to the prairie communities, which thrived when natural disturbances were altered. Today, these grasslands have been reduced to 0.01% of what originally existed (Knapp and Seastedt 1986).

In the twenty-first century world, humans are becoming more conscious of the environment, leading to an increased appreciation of the protection and restoration of natural ecosystems. Tracts of abandoned agricultural lands are being plowed under and seeded with native plant species. These costly restoration projects are well intentioned; however, reconstructed prairies cannot match the diversity of the few remaining remnant grasslands (Cairns 1993, Knapp and Seastedt 1998). Predicting which plant species will become established in a restored community is tenuous, at best. The limited success is

often attributed to a lack of knowledge of how prairie plants interact (i.e. rules of assembly). Restoration ecologists spend much of their time and energy studying the visible, above-ground component (i.e., planting mixtures) of the prairie; however, an equally important component exists below ground (Miller 1997).

Ecology of Prairie Soils --Prairies are truly a root-driven ecosystem (Miller 1997). It is not uncommon for prairie plants to allocate 65% of their biomass towards root architecture (Hartnett and Fay 1998, Miller 1997). Although most prairie plant root systems are restricted to the top meter of soil, it is not uncommon for many perennial species to penetrate well over 3-m deep (Zajicek et al 1986). The biomass plants produce below-ground manifests itself as a dense, tightly-packed mat of intermingled roots. Directly surrounding the plant roots is an area of soil that is composed of a densely-packed group of organisms including bacteria, fungi, and invertebrates (Killham 1994). This area of soil is termed the rhizosphere and is of great ecological significance for both plants and microbes. Exudates from the root provide a source of vitamins and nutrients for soil micro-organisms (Killham 1994). In turn, soil micro-organisms impact the plant, causing large shifts in plant nutrient uptake ability in a number of different ways (Miller 1997). These microbes cause changes in root morphology and architecture, alter soil chemistry around the root, compete with plants for nutrients, and form symbiotic relationships (Hetrick et al 1998a, Killham 1994).

Mycorrhizal relationships are among the most important symbiotic relationships occurring in the rhizosphere. Mycorrhizae (Greek: "fungus-roots") occur between plant

roots and soil fungi in the Phylum Glomeromycota. Mycorrhizal associations are widespread among most plant phyla and are thought to have evolved and spread with the earliest land plants over 400 Mya (Allen 1991, Simon et al 1993, Smith and Read 2008). Fungal colonization of early protorhizoids (rudimentary roots) may have been necessary in order for early plants to acquire nutrients from ancient soils (van der Heijden and Sanders 2002, Selosse and le Tacon 1998).

Arbuscular mycorrhizal fungi (AMF) are a relatively small group of fungi that are obligately symbiotic. The fungi are so named by the arbuscule (Greek: "little trees"), a fungal structure that is produced within root cortical cells that serves as an exchange organ between fungus and host (Allen 1991, Smith and Read 2008). Plants provide mycorrhizal fungi with carbon from photosynthesis, as much as 20% of total carbon is allocated towards their mycorrhizal partner (Allen 1991, Jakobsen and Rosendahl 1990). Plants receive considerable benefit in return, gaining access to poorly mobile, limiting nutrients such as phosphorus (Allen 1991, van der Heijden 2002, Miller 1997). The fungus forms a finely branched mycelial network extending outward into the rhizosphere. Production of extensive mycelial network allows the fungus to penetrate minute soil spaces, gaining access to nutrients locked inside of soil particles that were unavailable to the plant root on its own (Allen 1991).

Plants that harbor mycorrhizal fungi often display improved plant nutrition (Koide 1991), enhanced productivity (Hart and Klironomos 2002, van der Heijden 1998a), improved water relations (Allen 1991, Smith and Read 2008), improved tolerance to extremes in soil pH (Gibson 2009), tolerance to toxic heavy metals

(Gibson 2009) and reduced plant disease (Newsham et al 1994, Sharma et al 1992).

Mycorrhizal fungi also enhance soil aggregation and improve soil structure

(Miller and Jastrow 2000). Mycorrhizal relationships are especially important in areas

with nutrient-limited soils; in these soils, plants often display heavier rates of root

colonization and increase carbon allocation to their fungal partner (Jakobsen et al 2002,

Johnson et al 2006). Soils of the native tallgrass prairie were extremely nutrient

deprived, with plants in constant competition for available nutrients. Undisturbed

tallgrass prairies often support extremely diverse populations of mycorrhizal fungi, which

are highly active (Bentivenga and Hetrick 1992). In these ecosystems, it is common to

find 25 m of fungal hyphae within 1 g of soil, which scales to 20,000 km within a cubic

meter of soil (van der Heijden 2002, van der Heijden and Sanders 2002, Smith and Read

2008). Although plants are typically thought to receive benefit from mycorrhizal

relationships, all plants do not receive the same influence from fungal colonization. Plant

response to mycorrhizal fungi can be linked to the functional guild into which that

individual is placed.

Prairie Plant Communities -- Various prairie plants can be grouped into "functional

guilds" of species that share similar life form, life history, and ecological and

physiological traits (Brown 2004). As an example, many grasses are most active in

growth during the cooler months (cool-season grasses), and some are most active during

the warmer months (warm-season grasses) (Hetrick et al 1989a, 1989b). Within the

prairie plant community, there are four main functional guilds recognized by plant

ecologists: warm-season C_4 grasses, cool-season C_3 grasses, non-leguminous forbs, and the legumes. C_4 and C_3 grasses are so named because of differences in photosynthetic pathways. C_3 grasses undergo the Calvin-Benson Cycle which results in the creation of a 3-carbon sugar molecule, where as the C_4 grasses added a step to the Calvin-Benson Cycle which leads to the creation of a 4-carbon sugar molecule (Lambers et al 1998).

The tallgrass prairie is dominated by C_4 grasses like big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium scoparium*) and Indiangrass (*Sorghastrum nutans*) but are intermixed with sub-dominating members from the C_4 and C_3 grasses, as well as the legumes and non-leguminous forbs (Wilson and Hartnett 1998). Plants in these guilds have differential responses to mycorrhizal fungal colonization (Hartnett et al 1993, Hetrick et al 1989a, 1994, Wilson and Hartnett 1998).

Many of the C_4, warm-season grasses require mycorrhizal relationships for survival (Anderson et al 1994, Hetrick et al 1989b). In studies by Wilson and Hartnett (1998), three dominant warm-season grasses (*Andropogon gerardii*, *Schizachyrium scoparium*, and *Sorghastrum nutans*) showed upwards of a 99% dependence upon mycorrhizal fungi. Dependency was defined by Gerdemann (1975) as "the degree to which a plant is dependent on the mycorrhizal condition to produce its maximum growth or yield at a given level of soil fertility". Warm-season grasses have a coarser root system when compared to the cool-season C_3 grasses, which lends evidence to the increased dependence upon mycorrhizae (Hetrick et al 1988a). Generally, plants with coarse root systems will have a higher reliance upon mycorrhizal fungi than plants with finer roots; the bulky roots of warm-season grasses are not able to adequately remove

enough nutrients from small soil spaces to meet its demand (Gibson 2009, Jakobsen et al 2002, Smith et al 2000a). Thus, mycorrhizal dependency of plant species may be linked to its rooting architecture.

Although many C_4 grasses may require mycorrhizal relationships, most cool-season, C_3, grasses (*e.g.*, *Bromus inermis*, *Elymus cinereus*, *Fescuta arundinacea*, *Koeleria pyranidata*, *Lolium perenne*) are facultatively mycorrhizal and receive minimal, if any, benefit from mycorrhizal symbiosis (Hetrick et al 1988b, 1993). Cool-season C_3 grasses have finely-branched, fibrous root systems (Wilson and Hartnett 1998). Because of the cool-season grasses' rooting strategy, they are better able to obtain nutrients without the aid of mycorrhizal fungi than C_4 grasses (Hetrick et al 1988a).

Forbs are dicotyledons that differ in their ability to form mutualistic associations with nitrogen-fixing soil bacteria collectively known as rhizobia. Rhizobia include members from five genera of soil dwelling bacteria. Forbs that have the ability to enter into these rhizobial relationships are referred to as legumes. Forbs that cannot enter into these relationships are referred to as non-leguminous forbs. Legumes are generally considered to have a strong reliance upon mycorrhizal fungi, but many of the non-leguminous forbs display a variable range of reliance which is linked to its root fibrousness (Hartnett et al 1994, Wilson et al 2001).

Relationship between Plant and Fungal Communities -- Because mycorrhizal symbiosis has differential impact on individual plant species by altering the resource acquisition ability of the plant, mycorrhizal fungi may also strongly influence plant-plant interactions

in tallgrass prairies (Allen 1991, Hartnett and Fay 1998). These plant-plant interactions can have community level effects that determine plant diversity in a large area (Zobel and Moora 1995). Thus, plant community structure may be linked to its underground symbionts.

Recent work has shown that plant and fungal communities may be interdependent. It has been demonstrated that plant diversity, community structure, and productivity are influenced by mycorrhizal fungi and mycorrhizal fungal community structure (Bever et al 2001, Gange et al 1990, Grime et al 1987, Hart and Klironomos 2002, Hartnett and Wilson 1998, van der Heijden et al 1998b, 2006, Klironomos 2000, Sanders 2002). Furthermore, van der Heijden and colleagues (1998a) found that plants which depended heavily upon mycorrhizal fungi were differentially impacted by various mycorrhizal fungal species. Thus, both species identity and composition of mycorrhizal fungal communities may influence plant community structure (van der Heijden 1998a, Sheublin et al 2007), with certain fungal species providing more growth benefits to host plants than others. Although fungal species may differentially influence plant species, most fungal species can colonize the majority of plant species (Hart and Klironomos 2002, Klironomos 2000). In turn, most plants will host several fungal species concurrently (Allen 1996).

Approaching a similar idea, but from the fungal point of view, Bever and colleagues (1996) found that fungal diversity may be linked to the diversity of the plant community. Bever and colleagues found that fungal sporulation (Bever et al 1996, 1997) as well as fungal growth rates (Bever 2002) were host dependent. Host-dependent

differences also were observed in fungal colonization within plant root tissues

(Johnson et al 2003a, Vandenkoornhuyse et al 2002, 2003). Because sporulation of

mycorrhizal fungi is highly correlated with growth rate (Abbott and Gazey 1994),

increases in fungal growth rate will be associated with increases in sporulation.

Differences in fungal growth rates may be manifested as shifts in fungal spore

community composition and thus plant community (Bever 2002, Bever et al 2002).

Mycorrhizal fungal community structure is also driven by factors other than host

plants and host plant preference. Fungal species distribution is driven by a number of soil

factors; however, most soil factors impacting fungal distribution are influenced directly

or indirectly by the plant community, which include: organic matter, pH, soil moisture,

nutrient status, phenolic compounds, and other rhizosphere microorganisms

(van der Heijden et al 2006, Killham 1994). Mycorrhizal fungal communities are not

ubiquitous across an ecosystem but exist in patches, which are driven by the factors

above, along with plant hosts (Edgerton-Warburton and Allen 2000, Ernst et al 1984,

Hayman and Tavares 1985, van der Heijden 2002, Louis and Lim 1988,

Miller and Bever 1999, Stahl and Smith 1984).

Fungal community composition in many habitats can be drastically altered with

small changes to soil structure or nutrient availability (Johnson et al 2006). Previous

work has shown that fungal communities are reduced, in terms of species diversity and

sporulation levels, by common agricultural practices such as tillage and fertilizer

treatments (Miller and Jastrow 1992). Long-term implementation of these agricultural

practices can reduce fungal populations below the threshold needed to support highly dependent and diverse native plant communities (Brundrett 1990).

The sensitivity of complex systems to slight perturbations has profound implications for anthropogenic effects in tallgrass prairie ecosystems. Thus, the loss and gain of fungal and plant species within a community may have dramatic implications for plant community and overall ecosystem stability and processes (Bever et al 2002, Hart and Klironomos 2002, Vogelsang et al 2006). This may have consequences for both the direction and implementation of our restoration and management strategies. It will be difficult to restore and maintain a diverse plant community if we have overly degraded the soil microbial community and vice versa. Therefore, strategies for restoring native plant communities on previously-tilled land must account for the mycorrhizal fungal community which may or may not be present.

Impact on Plant Community Assembly -- Understanding the "rules" by which prairie plants assemble themselves requires an understanding of how mycorrhizal fungi influence plant establishment and productivity. "Assembly Rules" have been adopted to describe processes that facilitate or regulate the assembly of communities from species pools (Diamond 1975, Keddy and Weiher 1999). Assembly rules which can be biotic or abiotic; can be thought of as filters that allow incorporation of some species into the community but prevent others. Because our knowledge of how mycorrhizal fungal community influence plant ecology and coexistence is in its infancy, (van der Heijden and Sanders 2002), identifying specific rules guiding the assembly of prairie communities

is premature. The goal of this thesis research was to examine the significance of fungal community diversity as an influencing factor in the establishment and maintenance of complex prairie plant communities. My work also demonstrates that ecological patterns seen above-ground are at least partly driven by below-ground interactions. By gaining a better understanding of these below-ground factors, we can understand the complexity of these relationships and processes and their impact on the above-ground plant community. This would allow restoration ecologists to improve techniques to rebuild healthy, diverse prairie communities, as were once described by the first explorers (Kline 1997).

CHAPTER II

MYCORRHIZAL FUNGAL COMMUNITY STRUCTURE

IN A MANIPULATED PRAIRIE

ABSTRACT. Most natural plant communities support a diverse assemblage of arbuscular mycorrhizal fungi (AMF). These mutualistic fungi impact the biomass production, survivorship, and competitiveness of many plant species. AMF communities have the potential to affect plant community structure and vice versa. We examined AMF sporulation in an 8.1-ha reconstructed prairie in Eau Claire County, Wisconsin. In fall 2003, the site was planted with combinations of native prairie species from each of four functional guilds: C_3 grasses, C_4 grasses, legume, and non-leguminous forbs. The various seeding treatments were arranged in a completely randomized design; each treatment was replicated five times. We hypothesized that diverse plant seeding mixtures would promote AMF diversity. To further test the interaction between the plant and fungal communities, each plot was subdivided and treated with the fungicide chlorothalonil to suppress AMF, or ammonium nitrate fertilizer. Fertilizers were added to mimic soils typical of prairie reconstruction with a long agricultural legacy as well as to negate the importance of nitrogen-fixing legumes. Fungicide and nitrogen fertilization were added as artificial filters (assembly rules) to determine if AMF and soil nutrition could impact community assembly and structure. Replicate soil samples were taken during the summers of 2004, 2006, and 2007 from each subplot. Spores of AMF were extracted, identified to species, and enumerated.

Surprisingly, seeding treatment did not significantly affect fungal diversity and spore abundance. Ordination analysis over all 3 sample years revealed a strong impact of time on the AMF community. Slope and elevation of each plot also had strong influence on the community. In 2007 (4 years after initial treatment), fungal species richness was significantly decreased by both nitrogen and fungicide treatments as compared to the untreated plots. Multivariate analysis of variance revealed significant changes in the community due to both nitrogen and fungicide. Fungal species richness was positively correlated with plant productivity and plant richness. Thus, the fungal community mirrored patterns observed in the plant community. Plant and fungal communities appear to be related at some level, even though initial seeding treatment did not measurably affect the fungal community. Perhaps the time scale of this experiment (4 years) was not sufficient for the plant species composition to alter the fungal composition. As the plant community becomes more established, it may have more influence on the fungi.

Introduction

The advantages that arbuscular mycorrhizal fungi convey upon an individual plant have been well documented: increased growth, improved water relations, and improved disease tolerance (Hetrick et al 1988a, 1994). These fungi also can impact competitive relationships, community composition, diversity, and productivity of the supporting plant community (Allen and Allen 1984, Bever 2002, Fitter 1977, Hart and Klironomos 2002, Hartnett et al 1993, Hartnett and Wilson 1999, van der Heijden et al 1998a, 1998b, Scheublin et al 2007). Thus, mycorrhizal fungi may have impacts upon ecosystem

functioning and processes where they occur. Furthermore, several authors have found differential responses of the plant host depending on the identity of the fungal partner (van der Heijden 1998a, Scheublin et al 2007), with some fungal species or strains providing more growth benefit than others (van der Heijden 2002).

Though mycorrhizal fungi are ubiquitous in prairie ecosystems, not all plants benefit equally from the symbiosis. Various prairie plants can be grouped into "functional guilds" of species that share similar life form, life history, and ecological and physiological traits (Brown 2004). Within the prairie plant community, there are four main functional guilds recognized by plant ecologists: cool-season C_3 grasses, warm-season C_4 grasses, non-leguminous forbs, and the legumes. Most C_4 grasses and legumes are obligately dependent upon mycorrhizal fungi; however, most C_3 grasses show limited to no response to mycorrhizal fungi (Gibson 2009, Hartnett et al 1993, Hetrick et al 1988a, 1994). Mycorrhizal dependence of non-leguminous forbs is often linked to their root branching patterns, with more coarse root systems, having a greater reliance upon mycorrhizal fungi (Gibson 2009, Hartnett and Wilson 2002, Hetrick et al 1992, Jakobsen et al 2002, Smith et al 2000b).

Because mycorrhizal symbiosis has differential impacts on individual plant species and functional guilds by altering the potential resource acquisition of the plant, mycorrhizal fungi may also strongly influence competitive interactions, survivability of individuals, and composition of plant communities, which ultimately impacts plant community assembly (Hartnett and Fay 1998).

Plant community ecologists have strived for years to understand how natural plant community assemble themselves from species pools (Keddy and Weiher 1999, Wilson 1999) i.e., how are communities built from a certain set of raw materials? The term "assembly rules" was adopted by Diamond (1975) to describe this very problem (Keddy and Weiher 1999). Assembly rules are built upon finding and describing patterns among plant communities but more importantly the who, what, where, why, and how in mechanisms driving these patterns (Keddy and Weiher 1999). Though there are many observable patterns within plant communities, finding and describing the assembly rule(s) driving observable patterns is difficult and poses a challenge to plant ecologists (Keddy and Weiher 1999, Wilson 1999). However, because assembly rules seek to describe the patterns that are driven by interactions between species (*e.g.,* competition) (Wilson 1999), processes that influence plant-plant interactions, such as mycorrhizal symbiosis, must be accounted for. Because most assembly rules studies fail to account for underground processes, this may represent the difficulties associated with describing assemble rules in plant communities. Thus, understanding the "rules" by which prairie plants assemble themselves requires an understanding of how AMF influence plant establishment and productivity.

Examination of AMF spore communities associated with plants in a reconstructed prairie was done. Sporulation was analyzed because spores provide the only way to morphologically differentiate between AMF species, and gives an indication of what AMF species are thriving within that community. The reconstructed prairie was experimentally manipulated by varying initial plant seed mixtures. Initial seeding

mixtures were manipulated because seedling recruitment into communities is an important factor that determines plant diversity and to determine the role that mycorrhizal fungi play in plant establishment (Tilman 1997, Grubb 1977). Several authors have suggested that mycorrhizal fungi promote seedling establishment by incorporating emerging seedlings into the hyphal network (Francis and Read 1994, van der Heijden 2002, Zobel et al 1997). To further understand the link between plant and fungal communities, fungicide was used to suppress AMF. Suppression of fungi was performed to determine the role of AMF in plant community structure. Fertilization was used as an artificial filter to determine the impact of soil nutrition levels on both fungal and plant communities because most prairie reconstruction occurs in areas with a long agricultural history. This study was the first step to address how mycorrhizal fungi impact above-ground plant community assembly, establishment, and maintainance. The project was performed in collaboration with Professor Evan Weiher, a plant community ecologist at University of Wisconsin Eau Claire and Professor Tali Lee, a plant physiologist from University of Wisconsin Eau Claire. It was hypothesized:

H_1: Diverse plant seed mixtures will promote mycorrhizal fungal diversity.

H_2: AMF community structure will be influenced by fertilization and fungicide treatments.

H_3: Fertilization and Fungicide treatment will negatively influence soil inoculum potential.

H_4: Observed influence on fungal community structure will be mirrored by changes in the plant community.

Material and Methods

Study Site

The study site is an 8.1-ha (20 acre) parcel of private land in Fall Creek (Eau Claire County), Wisconsin, USA (44º 42' N, 91º 19' W). Prior to reconstruction, the site was used to grow alfalfa and corn in rotation for about 40 years (Stefanski 2009). The soil at the site is a Seton Loam (Thomas 1977) and has a mean pH of 6.3 and 0.23% total nitrogen (N) (Stefanski 2009). The study site is classified as having a humid continental climate with warm summers (mean July temp = 21 C) and cold winters (mean January temp = -12 C) with an average annual precipitation of 800 mm (Wisconsin State Climatology Office, 2008; Stefanski 2009). During the 2003 growing season, the herbicide glyphosphate (Round-up, Monsanto Co., Marysville, Ohio) was applied three times to eradicate as much vegetation as possible. Existing plant litter was partially removed with a tractor-pulled tine rake.

Experimental Design

In fall 2003, the site was planted with combinations of native prairie species from each of the four prairie functional guilds. The various seeding treatments (Table 2-1) were arranged in a completely randomized design; each of the nine functional treatments was replicated five times in a nested design.

Table 2-1. Overview of planting design. (Each treatment was replicated five times).

Trt #	Initial # plant sp.	# Functional Guilds	# Warm-season grass species	# Cool-season grass species	# Legume species	# Forb species
1	6	2	3	3	0	0
2	10	3	3	3	4	0
3	14	3	3	3	8	0
4	14	3	3	3	0	8
5	18	4	3	3	4	8
6	22	4	3	3	8	8
7	22	3	3	3	0	16
8	26	4	3	3	4	16
9	30	4	3	3	8	16

An equal mass of seeds from each species was used to plant 538 seeds m^{-2} (Diboll 1997, Stefanski 2009). Seeds were mixed with vermiculite, and then large-seeded species were hand-tossed and raked in. Small-seeded species were similarly hand tossed and the plot was rolled. Planting was finished in early December 2003, prior to snow cover. All seeds were obtained from Prairie Moon Nursery (Winona, Minnesota).

The species pool (Table 2-2) included a mixture of common highly-productive species, less-productive species that were more tolerant to drought and soil extremes, as well as some climax species that are indicative of successionally advanced, high quality prairies (*e.g., Astralagus crassicarpus* (groundplum milkvetch) and *Gentiana alba* (Plain Gentian). All species used were indigenous to Eau Claire County, Wisconsin or an adjacent county except for *A. crassicarpus* (which is found 60 miles away in Pierce and St. Croix Counties). Each of the nine functional mixtures were replicated with random selections from the species pool, giving 45 individual unique seeding mixtures.

Table 2-2. Prairie species planted from each functional guild.

Functional guild	Plant Species
Warm-season grasses	*Andropogon gerardii, Bouteloua curtipendula, Sorghastrum nutans, Schizachyrium scoparium, Sporobolus heterolepis*
Cool-season grasses	*Bromus kalmii, Carex bicknellii, Carex brevior, Elymus canadensis, Koeleria macrantha, Stipa spartea*
Legumes	*Amorpha canescens, Astralagus canadensis, Astralagus crassicarpus, Baptisia alba, Dalea candida, Dalea purpurea, Desmondium canadense, Desmodium illinoense, Lespedeza capitata, Lupinus perennis*
Forbs (non-legumes)	*Asclepias tuberosa, Asclepias verticillata, Aster pilosus, Aster laevis, Aster novae-angliae, Aster oolentangiensis, Coreopsis palmata, Gentiana alba, Helianthus grosseseratus, Helianthus occidentalis, Heliopsis helianthoides, Liatris aspera, Liatris punctata, Monarda fistulosa, Monarda punctata, Ratibida pinnata, Solidago rigida, Verbena stricta, Veronicastrum virginicum, Zizia aurea*

To further test the interaction between plant and fungal communities, each plot was subdivided in a split plot, complete factorial design. After seeding, each 0.1-hectare plot was split into four subplots of 256-m^{-2} each. Ammonium nitrate fertilizer was applied three times annually to one-fourth of the subplots at a rate of 15-g N m^{-2} yr^{-1} in 2004, 2005 and 7-g N m^{-2} yr^{-1} in 2006. Nitrogen fertilizers were used to mimic the high nitrogen content typical of prairie reconstruction a soil with a long agricultural legacy as well as to negate the impact and importance of nitrogen-fixing legumes. One-fourth of the plots received fungicide application to suppress mycorrhizal fungi within subplots. The fungicide chlorothalonil (tetrachloroisophthalonitrile) was applied three times throughout the growing season at annual loading rates of 18-g m^{-2} yr^{-1} in 2004, 12-g m^{-2}

yr^{-1}in 2005 and 6-g m^{-2} yr^{-1} in 2006. One-fourth of the subplots received both nitrogen and fungicide treatment, and one-fourth remained untreated (control). Although the project began in 2003, my research began in the 2006 sampling season.

Sample collection and processing

In July 2004, 2006, and 2007, soil samples were collected from each subplot. Ten replicate subsample soil cores (2.5-cm x 15-cm) were taken from randomly selected locations within each subplot. These subsamples were then mixed together and transported back to the lab at UW-Oshkosh and stored at 4 C.

Approximately one-third of each soil sample was used to quantify the mycorrhizal fungal taxa present. Spores were extracted from 100-ml subsamples of soil using the method of Daniels and Skipper (1982). Soil was placed in a 32-μm mesh sieve and washed vigorously with cold tap water. Spores were then separated from larger soil particles by placing samples within a 60% sucrose solution and centrifuging for three minutes. The cleaned spore preparation was examined microscopically, and fungal spores were identified to species and enumerated. Spore identification was done in 2004, by D.L. Badtke-Shamsi, and identification and counts were done in 2006 and 2007 as part of this thesis.

To determine the mycorrhizal inoculum potential (MIP) of Eau Claire soil, 100-ml of each subsample was taken and mixed 1:1 (v/v) with sterile sand and placed in 4 x 20.5-cm "conetainers" (Stewe and Sons, Corvallis, Oregon.). Seeds of sorghum-sudangrass hybrid (variety Super Sv 22, purchased from Olsen's Mill, Auroraville, Wisconsin) were surface sterilized in 5% bleach for 20 minutes and were pre-germinated

in Turface Proleague™ (Buffalo Grove, Illinois) and were then transferred to "conetainers" at 2 weeks of age. Seedlings were watered daily and were grown in the greenhouse at UW-Oshkosh (15–25 C) for 28 days.

At 28 days, plants were harvested and the washed root systems of sorghum plants were collected. A 0.5-g sample of each root system was clipped to 2-cm sections, cleared, and stained with trypan blue (Koske and Gemma 1989). Stained roots were examined microscopically for evidence of mycorrhizal colonization using the "grid-line intercept" method (Giovannetti and Mosse 1980). Measuring colonization allowed estimates of the percentage of root material that was occupied by mycorrhizal fungi and how effective each subplot's mycorrhizal community was at colonizing a potential host plant (inoculum potential). Although sorghum-sudangrass is not a native prairie species, it is commonly used to estimate inoculum potential. Mycorrhizal inoculum potential data were collected in 2006 and 2007.

Plant productivity was measured each fall by Evan Weiher's group at UW-Eau Claire. This was done by clipping above-ground biomass in a 0.25-m^{-2} quadrat in each subplot in early June, and again in September. Plant productivity is a measure of the amount of dry material that is obtained by integrating net primary production over a specified amount of time (Gibson 2009). The time from early June through September represents the main growing season within a prairie community and since grasses senesce after the growing season, represents the annual net primary production (NPP).

A census of plant species in each subplot also was taken in early September each year by Weiher's group. The census measured diversity of plant community and the relative abundances of plant species within the community.

Statistical Analysis

Absolute abundances (Figs. 2-2, 2-3, 2-4) of fungal species spore counts were subjected to multi-variate analysis of variance (MANOVA) to determine difference in fungal sporulation among the different treatments (nitrogen, fungicide, nitrogen and fungicide) for each fungal taxon. Frequency of occurrence (Figs. 2-5, 2-6) was calculated as: (number of subplots – subplots without sp.)/ (number of subplots). Fungal spore frequency of occurrence (Figs. 2-5, 2-6) was also subject to MANOVA to detect differences among species, associated with the differing treatments.

Total sporulation (all fungal taxa combined, Fig. 2-2) and fungal species richness (Fig. 2-8) were analyzed by univariate analysis of variance (ANOVA) to determine differences among treatments. MANOVA was also applied to several diversity indices (Shannon-Weiner Index, Simpson's Diversity Index, Fisher's Alpha (Fig. 2-8)) within each of the subplot treatments, which analyzed richness levels, number of individuals, diversity measures, and evenness measures. All analyses were conducted for each year separately as well as combined. Combined data (Figs. 2-2, 2-7, 2-8) were further subjected to Tukey's HSD procedure to determine differences between the sampling years. Mycorrhizal inoculum potential data (Fig. 2-9) for 2006 and 2007 also were subjected to univariate ANOVA to determine differences among plot treatments.

Non-metric multidimensional scaling (MDS) (Figs 2-10, 2-11) was also used to assess treatment effects on the fungal spore community. MDS was used on 2007 spore community data (Fig. 2-10) as well as the 3-year combined data (Fig. 2-11). MDS was used to elucidate patterns that may not have been obvious using ANOVA and MANOVA. Data from 2007 were analyzed using the Morisita similarity index and Jaccard's similarity index. The Morisita index measures (Fig. 2-10) how similar or dissimilar the communities are based on species abundances and Jaccard's index more heavily weights presence/absence of species.

These analyses were performed on the subplot (split plot) level of the fungal community, to test how treatments (nitrogen, fungicide, both) were impacting fungal community structure. Three year combined MDS (Fig. 2-11) used Euclidian distance to explore how fungal community structure had changed over time. In addition to the treatments, environmental factors such as slope and elevation were analyzed to determine if they had measureable impact on fungal community structure.

To examine the relationship between plant and fungal communities, Pearson Product-Moment Correlation Coefficient was used on measures of fungal community (our data) and plant community (Weiher's data) (Figs. 2-12, 2-13). Community measures used in regressions were: plant and fungal richness, fungal spore abundance, percent root colonization, plant productivity, and plant biomass production.

Results

Through the 2 sampling years, a total of fifteen species were identified, which represented five different fungal genera (Table 2-3). In 2006, all fifteen species were observed; however in 2007, sporulation of *Glomus* sp. (clear outer) was not observed.

Table 2-3. Fungal species observed
Entrophospora infrequens
Gigaspora albida
Gigaspora gigantea
Gigaspora margarita
Gigaspora rosea
Glomus aggregatum
Glomus clavisporum
Glomus sp. "clear outer"
Glomus constrictum
Glomus etunicatum
Glomus intraradices
Glomus mosseae
Paraglomus occultum
Scutellospora calospora
Scutellospora pellucida

Surprisingly, nested ANOVA revealed no significant impact of the initial seeding mixtures on the mycorrhizal fungal spore community (Fig. 2-1), which led us to reject our first hypothesis: diverse plant seed mixtures will promote fungal diversity.
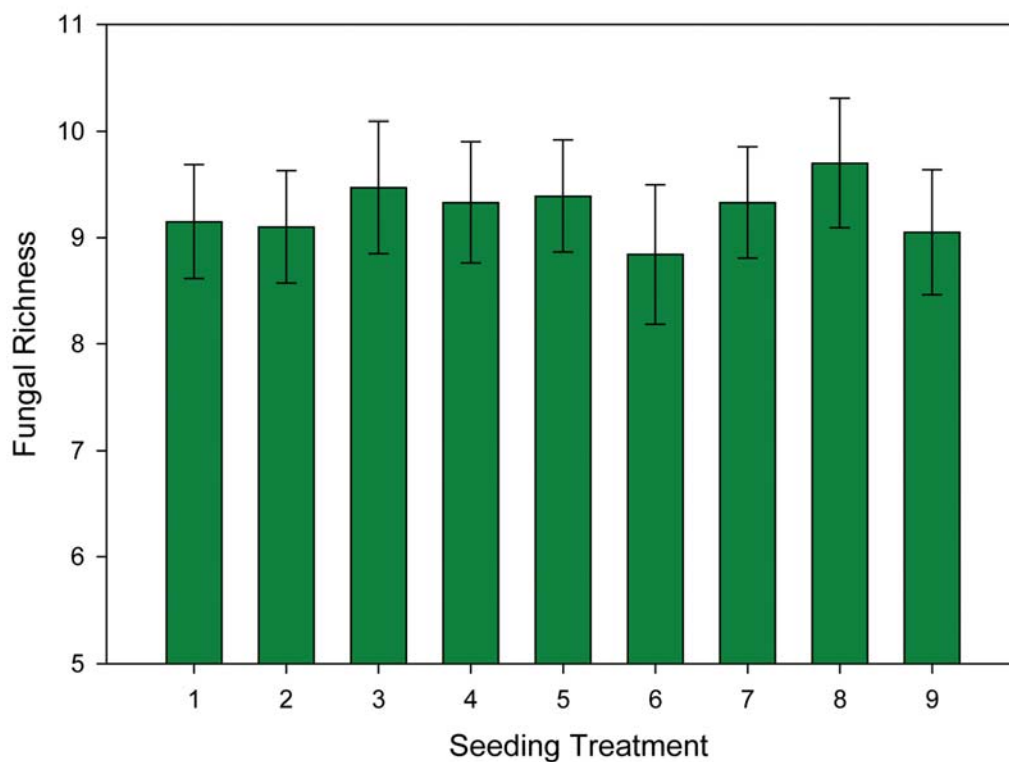
Figure 2-1. AMF richness per differing plot seeding mixtures in 2007. Richness

increases from left to right. Refer to Table 2-1. Error bars represent 95% confidence

intervals.

Work by Badtke in 2004 revealed no differences in soil treatments (fungicide and

nitrogen) on mycorrhizal species in the subplots in the first year after initial setup

(pers. comm.). However, significant impact of nitrogen and fungicide was observed

within the fungal community in both 2006 and 2007, supporting H_2. The influence of

nitrogen and fungicide became more pronounced throughout the sampling years.

Total sporulation in our subplots increased significantly from 2006 to 2007 (Fig. 2-2). Sporulation increased among all treatments between sampling years; however the most dramatic increases were observed in the control plots, increasing from 760 spores per 50-ml soil sample in 2006 to 1060 spores per sample in 2007 (Fig. 2-2). In both sampling years, fungicide-treated subplots displayed increased sporulation (Fig. 2-2). Nitrogen fertilization, which had no impact on subplots in 2006, resulted in reduced sporulation in 2007 (Fig. 2-2).
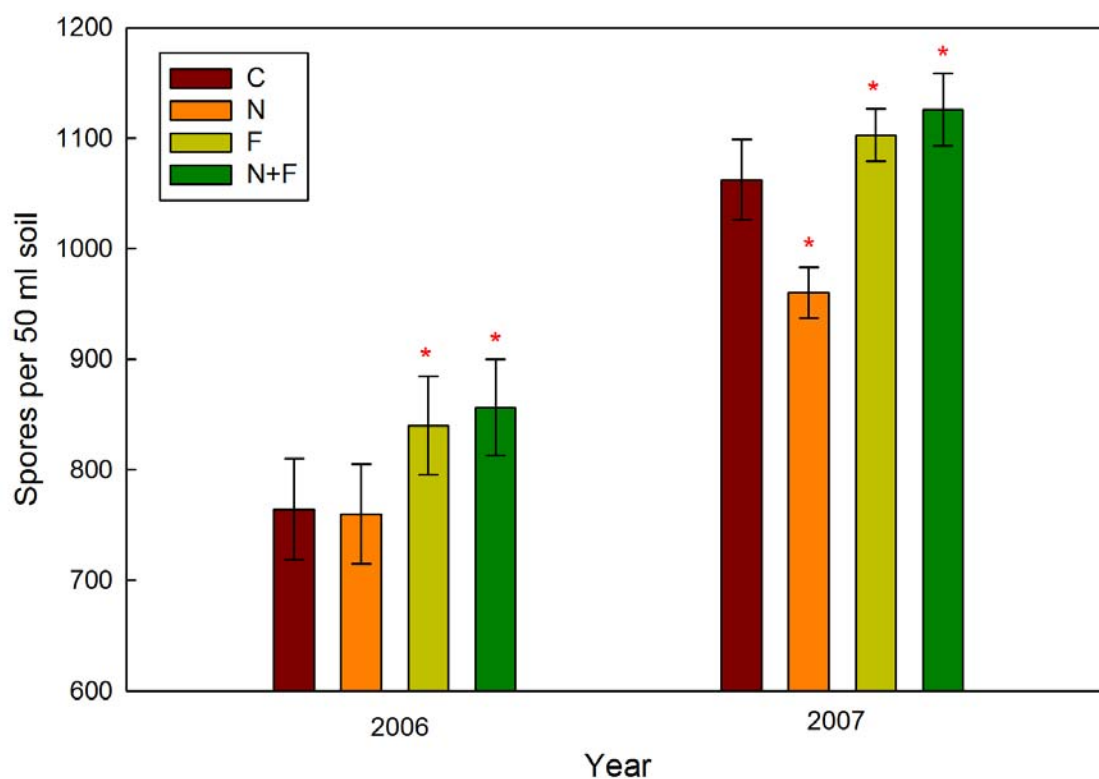
Figure 2-2. Abundance of AMF spores, as influenced by treatment across 2 sampling years. Means with asterisks differ significantly from the control, according to MANOVA. Error bars signify a 95% confidence interval.

Increased sporulation in fungicide subplots was unexpected; however, sporulation

appears to be driven by *P. occultum* (Figs. 2-3, 2-4). Sporulation of *G. etunicatum*

significantly increased in fungicide subplots in 2006 (Fig. 2-3), but not in 2007 (Fig. 2-4).

Other than *P. occultum* and *G. etunicatum*, application of fungicide lowered sporulation

of all other fungal species. Control plots in both 2006 and 2007 were dominated by *G.*

etunicatum, *G. constrictum*, and *G. aggregatum,* where as subplots with fungicide

application were dominated by *P. occultum*. The impact of fungicide on species becomes

more pronounced in 2007 when compared to 2006 (Figs. 2-3 and 2-4).
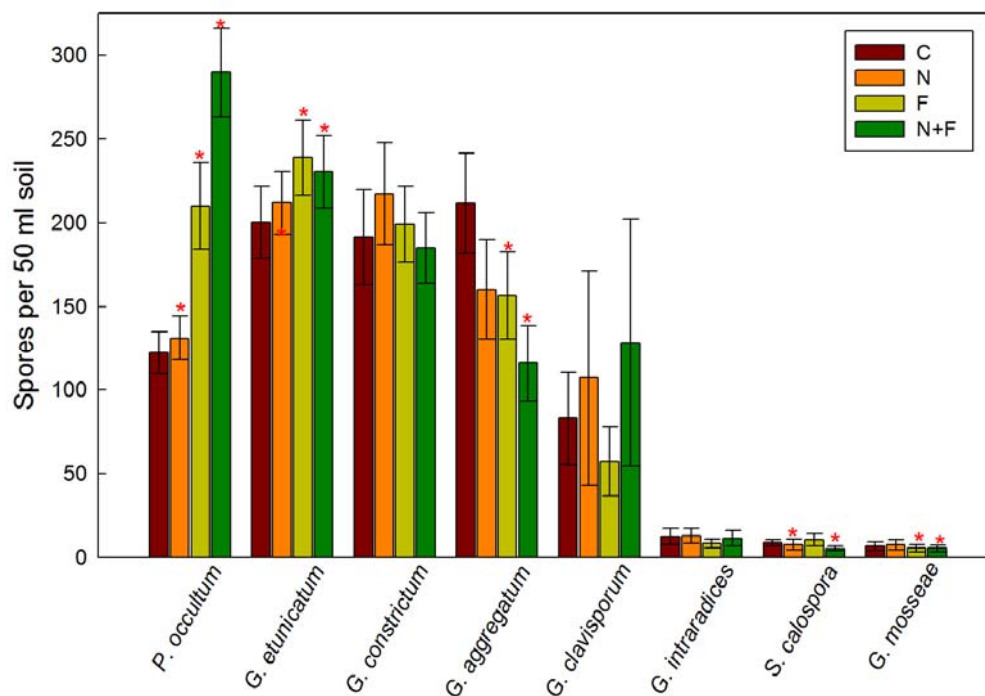
Figure 2-3. Abundance of AMF spores of AMF species in 2006, as influenced by
treatment. Within a species, means with asterisks differ significantly from the control,
according to MANOVA. Error Bars represents 95% confidence intervals.
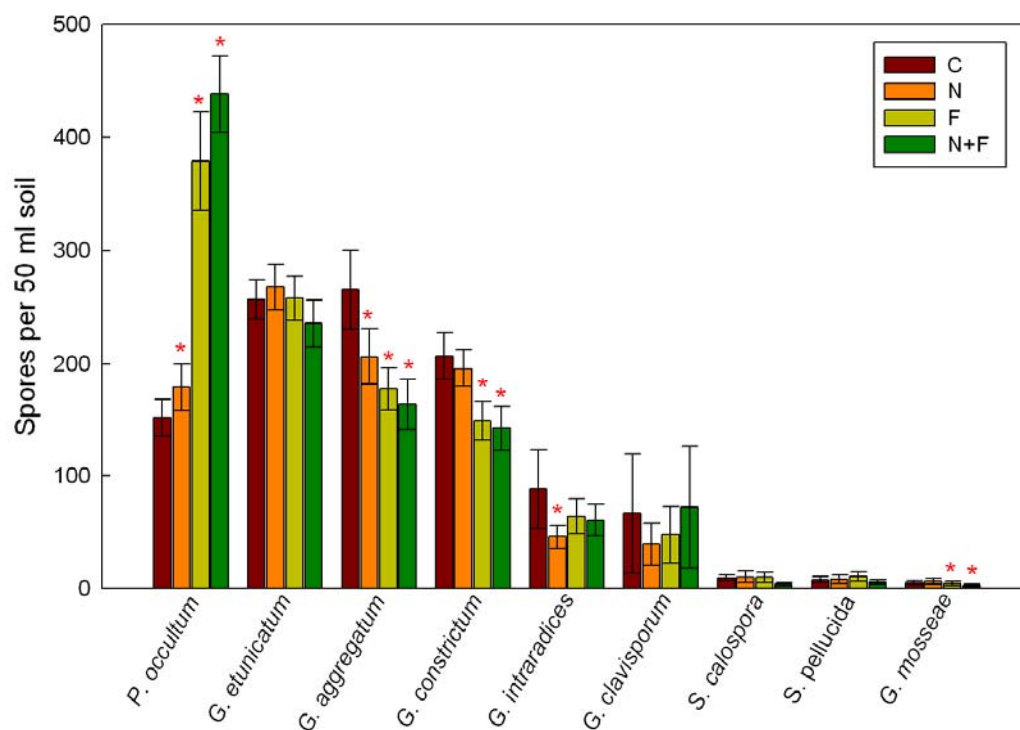
Figure 2-4. Abundance of AMF spores of AMF species in 2007, as influenced by treatment. Within a species, means with asterisks differ significantly from the control, according to MANOVA. Error Bars represents 95% confidence intervals.

Analysis of frequency of occurrence revealed that in 2006 only two species were found in all subplots (*G. constrictum* and *G. etunicatum*), while *G. aggregatum* and *P. occultum* each were found in all but one subplot, respectively (Fig. 2-5). *S. calospora*, *G. intraradices*, *G. clavisporum*, *G. mosseae*, and *Gi. albida* were found in approximately 50% of subplots. Occurrence of *S. calospora* spores was reduced in nitrogen subplots, and occurrence of *G. clavisporum* spores was reduced in fungicide subplots (Fig. 2-5).
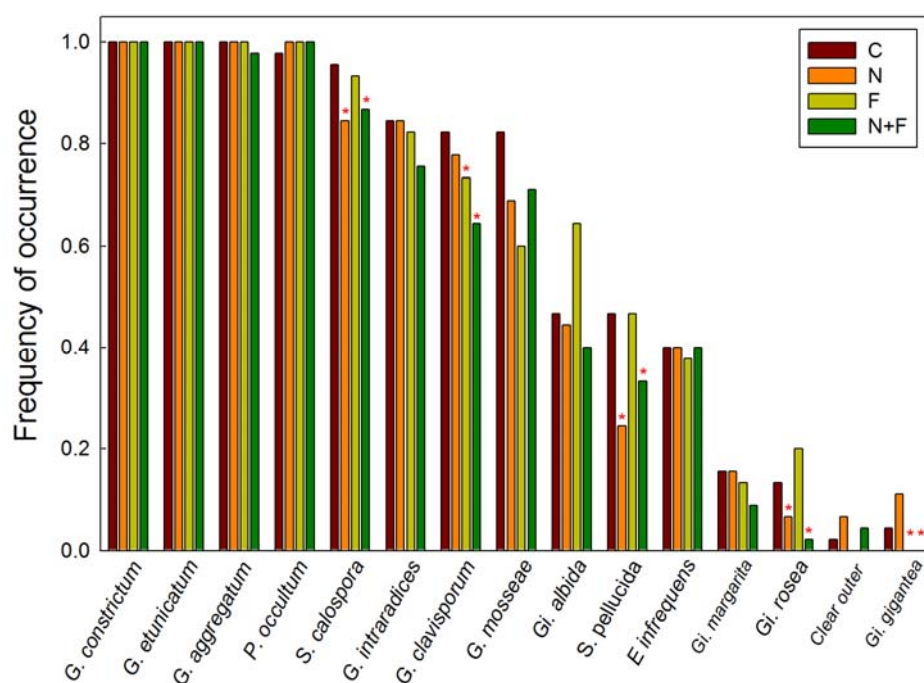
Figure 2-5. Frequency of occurrence of AMF spores by AMF species in 2006, as influenced by treatments. Asterisks indicate significant differences from control, according to MANOVA.

Although both species occurred less frequently in these subplots, they did not display

reduced spore abundances (Figs. 2-3, 2-4). *S. pellucida* and *E. infrequens* occurred in

approximately 40% of subplots. Occurrence of *S. pellucida* spores was reduced in

nitrogen subplots (Figs. 2-5, 2-3, 2-4). Frequencies of *Gigaspora* spores were reduced in

both nitrogen and fungicide subplots (Fig. 2-5).

In 2007, *G. constrictum*, *G. etunicatum*, *P. occultum*, and *G. aggregatum* occurred

in all subplots, as well as *G. intraradices*, which was only found in 80% of subplots in

2006 (Figs. 2-5, 2-6). Sporulation of *S. calospora* was similar in both years, however

sporulation of *S. pellucida* increased from 2006 to 2007 (Figs. 2-5, 2-6). The decline in

G. clavisporum spore abundance (Fig. 2-3, 2-4) was matched by a decline in occurrence

in subplots (Fig. 2-6). Frequencies of *Gi. albida*, *Gi. rosea*, and *Gi. gigantea* spores

increased in 2007, however, *Gi. margarita* showed a decline in occurrence compared to

2006.
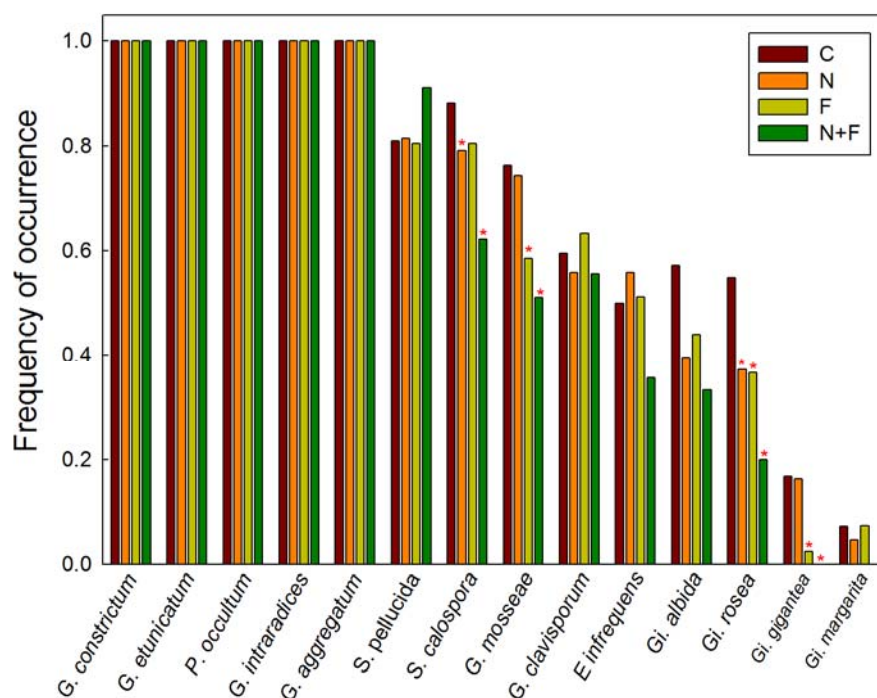
Figure 2-6. Frequency of occurrence of AMF spores by AMF species in 2007, as influenced by treatments. Asterisks indicate significant differences from control, according to MANOVA.

Aside from the observable changes in spore abundances and occurrence, community level diversity and richness shifts also were observed. Overall fungal richness (number of species per subplot) increased from 2006 to 2007 (Fig. 2-7). Richness was significantly reduced by nitrogen but not fungicide in 2006 and was reduced by both nitrogen and fungicide in 2007 (Fig. 2-7).
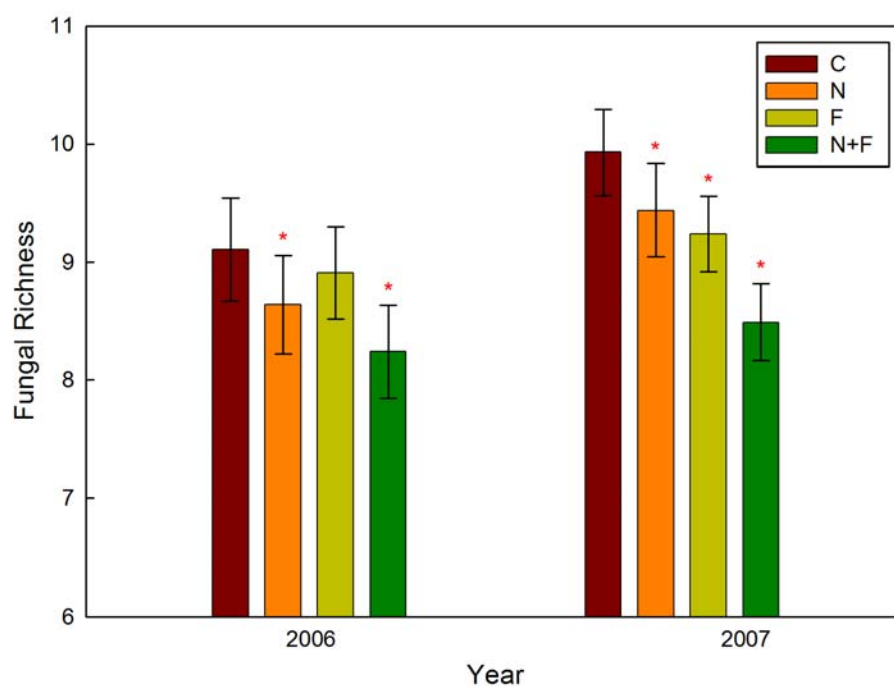
Figure 2-7. Detected AMF species richness per sample as influenced by treatment across 2 sampling years. Means with asterisks differ significantly from the control, according to MANOVA. Error bars signify a 95% confidence interval.

Other measures, such as Fisher's alpha diversity index (Fig. 2-8), Simpson's, Shannon-Wiener, and Margalef's (all not shown) revealed that diversity among subplots was significantly altered by nitrogen and fungicide treatment in both 2006 and 2007 (Fig. 2-8). Additional mycorrhizal fungal taxa were identified in 2006 and 2007 that were not present in 2004 samples, which led to significant increases in fungal richness levels (all treatments combined).
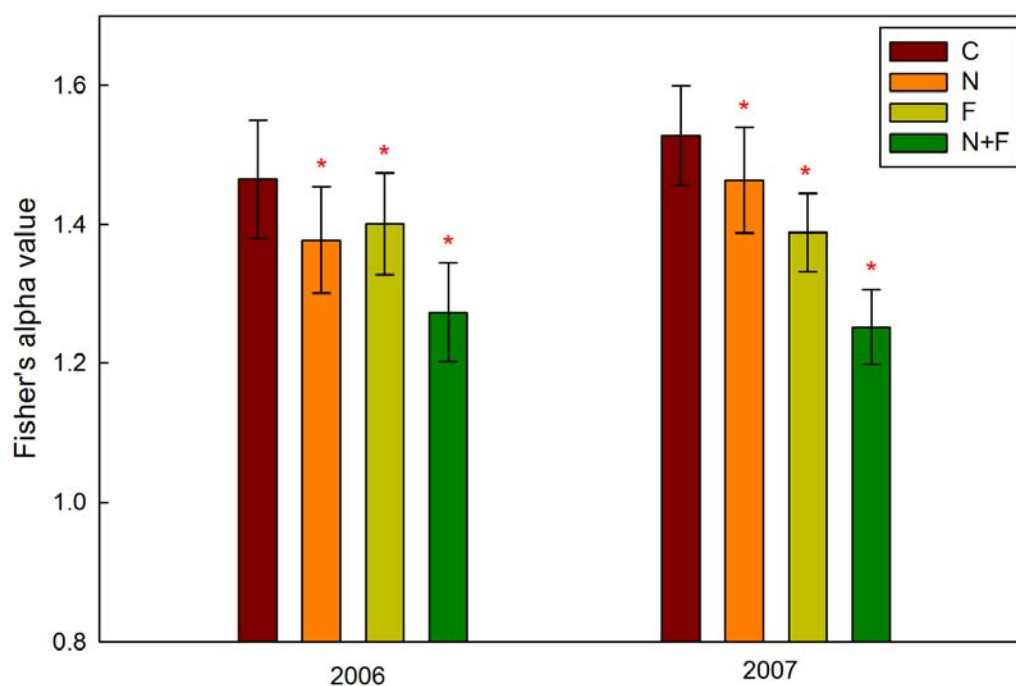
Figure 2-8. Calculated Fisher's alpha measurement of diversity per sample, as influenced by treatment across 2 sampling years. Within a year, means with asterisks differ significantly from the control, according to MANOVA. Error bars signify a 95% confidence interval.

Application of fungicide in subplots led to a reduction in inoculum potential of prairie soil in 2006 (Fig. 2-9), but, nitrogen application did not. Combined nitrogen and fungicide application led to reduced colonization potential, which was approximately 50 % of the control (Fig. 2-9). In 2007, MIP of control subplots doubled relative to 2006 (Fig. 2-9). In 2007, both nitrogen and fungicide reduced MIP, supporting H_3 (Fig. 2-9).
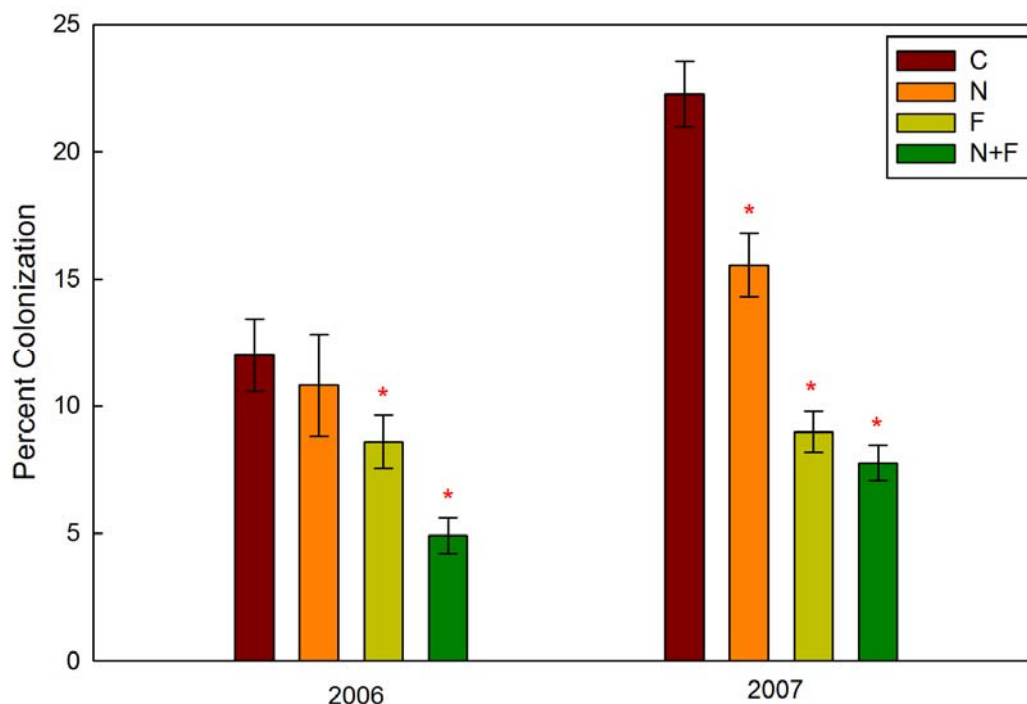
Figure 2-9. Mycorrhizal Inoculum Potential (MIP) per soil sample, as influenced by treatment across 2 sampling years. Means with asterisks differ significantly from the control, according to MANOVA. Error bars signify a 95% confidence interval.

Non-metric multi-dimensional scaling (NMDS) of 2007 spore data revealed that fungicide application caused measureable shifts in the mycorrhizal fungal community (Fig. 2-10). Interestingly, NMDS revealed no significant influence of nitrogen on the spore community composition (Fig. 2-10).
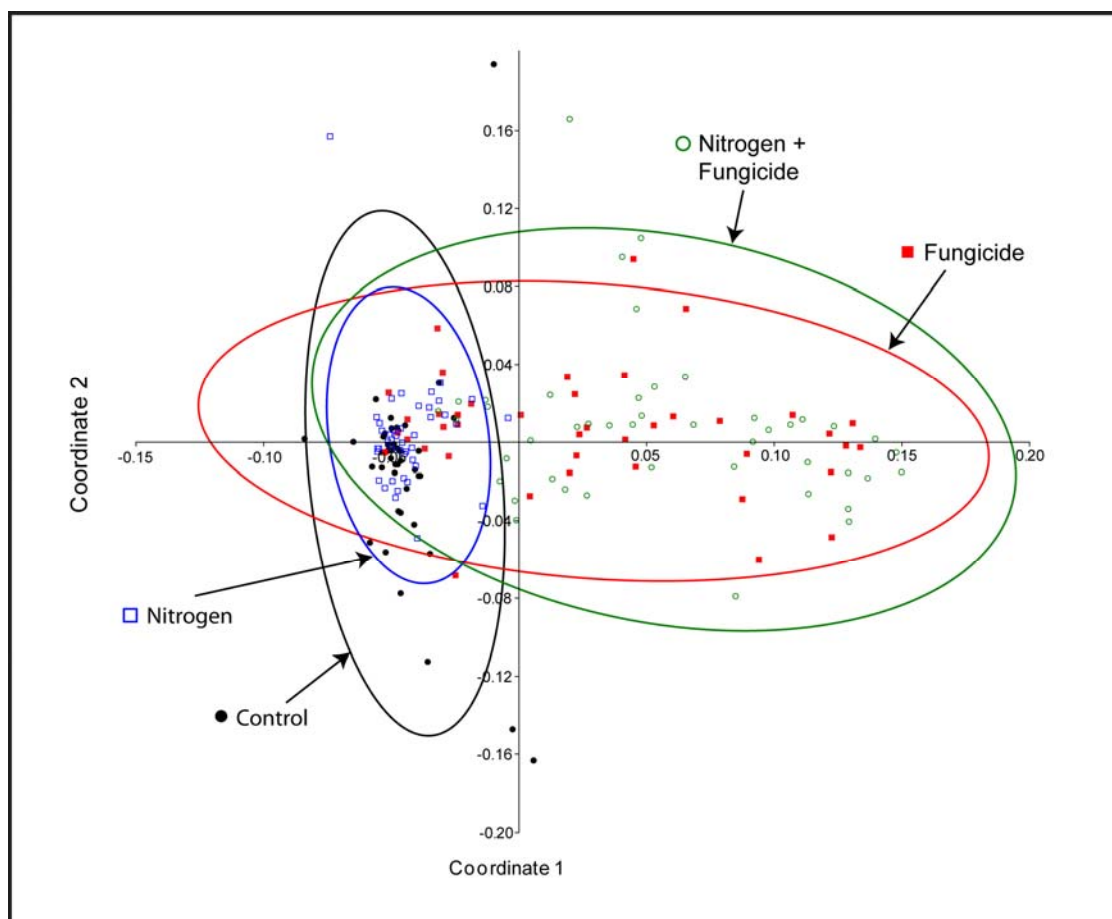
Figure 2-10. Non-metric Multi-dimensional scaling for 2007 spore data, as influenced by treatment using Morisita's Index. Ellipses represent a 95% confidence interval.

The NMDS of the 3-year combined fungal community with 2004 data collected by Badtke, revealed that the spore community had shifted significantly throughout the sampling years (Fig. 2-11). Much of the shift can be attributed to the presence of fungal species that were not present in 2004 sampling (*G. aggregatum*, *G. intraradices*, *G. clavisporum*, *S. pellucida*, *Gi. margarita*), as well as the large shift in abundance levels of

species that were already present (*P. occultum*). In addition to the influence of the soil

treatments, environmental factors such as slope and elevation also measurably impacted

the fungal spore community (Fig. 2-11).
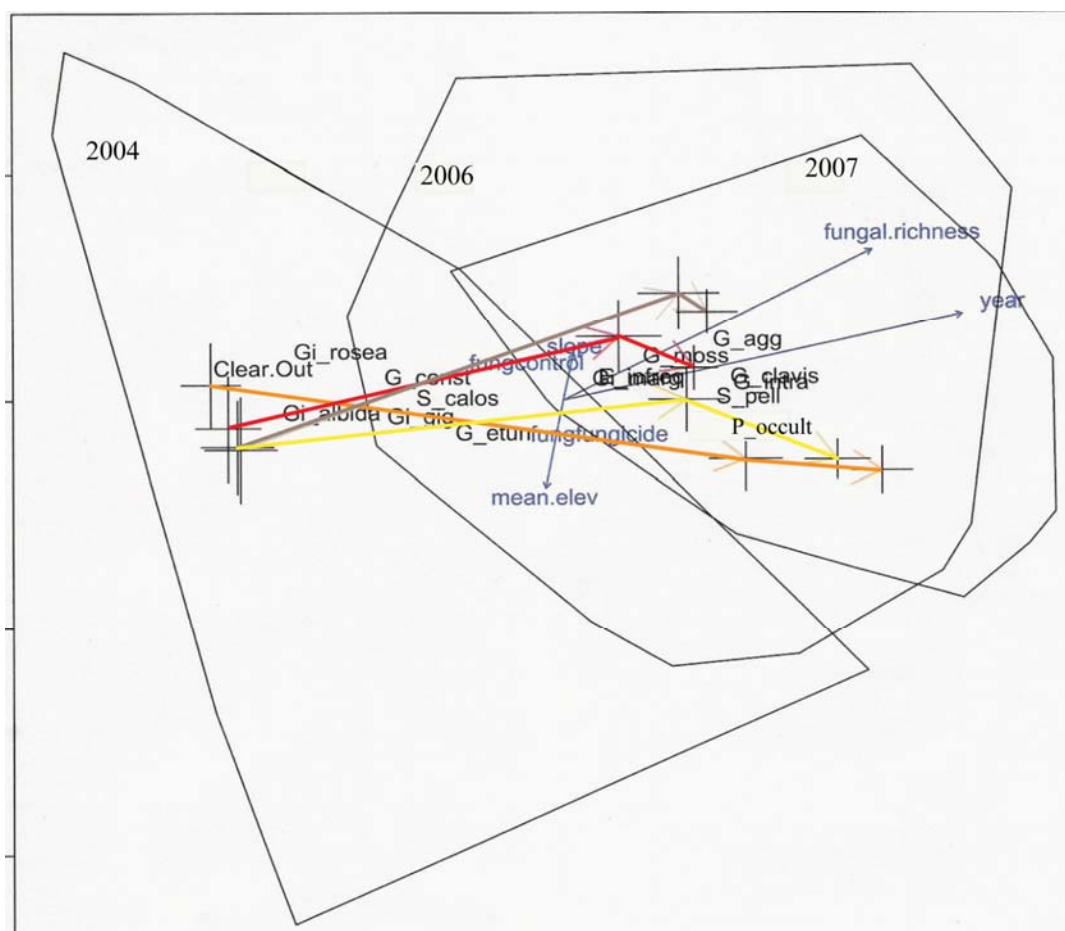
Figure 2-11. Non-metric Multi-dimensional scaling across 3 sampling years as influenced

by treatments and subplot elevation, subplot slope, year. Colored lines represent

treatment affect on the AMF community through the three sampling years (red =

nitrogen, yellow = fungicide, tan = control, and orange = nitrogen + fungicide). The

convex hulls surround the sampling year datasets. Figure by Evan Weiher. 2004 data

collected by D. Badtke.

Because the fungal community is intricately tied to the above-ground plant
community, it was necessary to incorporate fungal data with plant data collected by Evan
Weiher and Colleagues at UW-Eau Claire. Observed radiation in the fungal communities
was mirrored in the above-ground plant community, supporting H_4. Correlation analysis
revealed a significant relationship between fungal species richness and plant richness
(Fig. 2-12) and fungal species richness and plant productivity (Fig. 2-13) in 2007. In
2006, the relationship between plant richness and biomass production was not
significantly linked to fungal richness levels (data not shown).
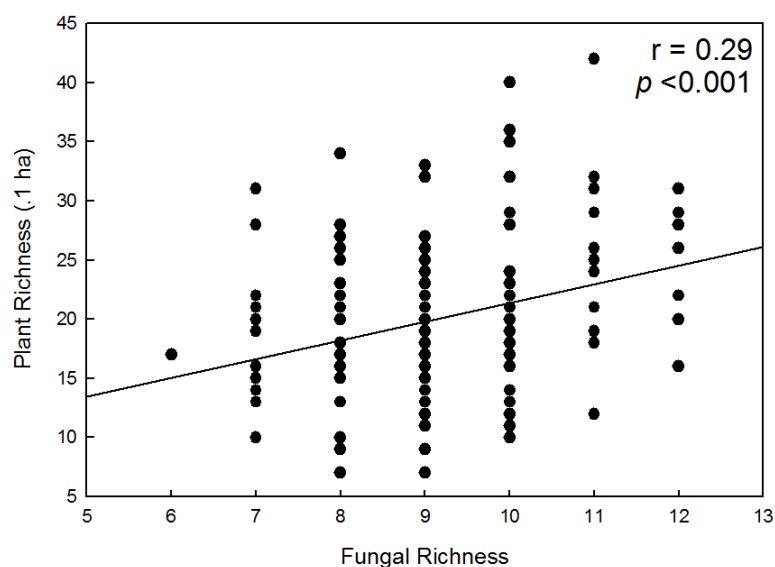
Figure 2-12. Pearson Product-Moment Correlation Coefficient of Fungal Richness per
AMF species and plant species richness per subplot for 2007.
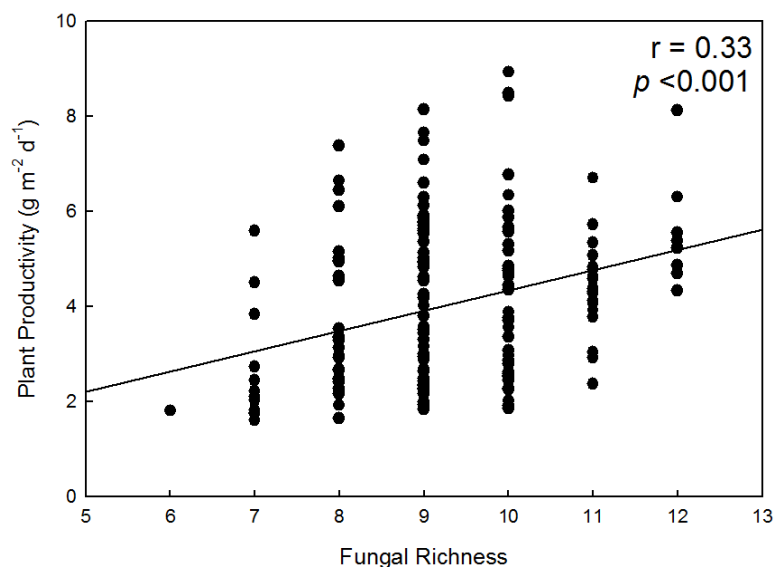
Figure 2-13. Pearson Product-Moment Correlation Coefficient of Fungal Richness per

AMF species and plant production measured in grams per m^{-2} per day^{-1}.

Discussion

Hypothesis 1: Most surprisingly, no relationship between initial seeding treatment and

mycorrhizal fungal diversity was observed, leading to the rejection our first hypothesis

(Fig. 2-1). This was surprising because many authors have found that mycorrhizal fungi

positively impact the establishment of seedlings into communities by incorporating

seedlings into hyphal networks and providing them with critical nutrients (Francis and

Read 1995; Zobel et al 1997; Van der Heijden 2002). To explain this observation, there

are a number of possibilities: 1) the experimental period (4 years) was not long enough

for feedback mechanisms between plant and fungal communities to become established.

2) Factors such as slope and elevation, or other natural factors impact plant and fungal

communities more so than does the initial seeding treatment. 3) When the prairie was established, the fungal community was at a dormant state and was not able to rebound until after suitable host plants were already established. This study did not allow us to assess any of these possibilities, so the mechanism behind the observation remains unclear. Long-term experiments are needed to determine whether initial seeding treatment will have an impact on fungal diversity once both communities become more established.

Hypothesis 2: In 2004, the first year after prairie reconstruction, Badtke observed a fungal community with no differences of sporulation patterns between nitrogen and fungicide applications. Although no differences in sporulation were observed in 2004, by 2006, nitrogen and fungicide were impacting richness and spore abundances within subplots supporting H_2.

In accordance with increases in fungal richness levels in subplots, levels of fungal sporulation also increased through sampling years. It appears that once suitable plant hosts became established, the underground fungal community responded with increased levels of sporulation. Surprisingly, sporulation was greatest within fungicide subplots (Fig. 2-2). The increase in sporulation was being driven by *P. occultum* within fungicide subplots, whereas most other species' sporulation was reduced (Fig. 2-4).

NMDS analysis revealed that the fungal community shifted significantly throughout sampling years. Much of the shift appears to have occurred because of additional taxa found in 2006 and 2007, and also the sporulation increases of *P.*

occultum. Also, environmental factors such as slope and elevation impacted the fungal community.

Although richness levels declined significantly in both nitrogen and fungicide treatment subplots, Jaccard's analysis revealed no significant differences in species presence/absence within the subplots (data not shown). Thus, divergence within the subplot communities was not being driven by the inclusion or exclusion of certain fungal taxa, but was being driven by shifts in species spore abundances in response to treatment within the subplots.

The response of *P. occultum* to fungicide treatment was not predicted. The mechanism behind *P. occultum*'s increase in sporulation is not known. Although no conclusions can be made from what caused increases in *P. occultum* sporulation, possible reasons could be hypothesized. One possibility could be the reduction in overall community-wide fungal growth rates allowed a semi-tolerant species to chlorothalonil to fill the open niches from the fungal decline. A second possibility was that hyphal growth of *P. occultum* along with the other fungal species were reduced by fungicide application, but the response of *P. occultum* was to markedly increase sporulation. Because DNA sequencing was not done on plant root material, composition of fungi that were actually colonizing roots was not determined. Therefore, determination could not be made whether sporulation increases were tied to increases in root colonization as reported by Bever (2002) or as in Wilson (1984), who found that increased sporulation was correlated with lowered competitive ability of the fungus.

It is also unknown if *P. occultum* provides any benefit to host plants because mycorrhizal fungi species do not have a uniform effect on all plant species (van der Heijden et al 1998b, Scheublin et al 2007). Although nearly all mycorrhizal fungal taxa may infect most plants, they may influence those host plants differentially. Because mycorrhizal relationships have various effects, different fungal taxa may be able to perform different tasks within plant hosts (Hart and Klironomos 2002). A broad continuum exists in which fungal impact on host plant can range from mutualism to parasitism (Hart and Klironomos 2002, Johnson et al 1997). Lab experience with *P. occultum* suggests that it is a species that is a common greenhouse contaminant and is considered to be a "weedy" fungal species. These observations lend evidence towards the hypothesis that the lack of host specificity of mycorrhizal fungal species does not mean functional redundancy, which was a long standing theory in plant and fungal ecology (Hart and Klironomos 2002, Read 2002).

Because productivity and biomass production was reduced in fungicide subplots, even though sporulation of *P. occultum* was significantly greater, it was possible that *P. occultum* was a growth- and productivity-inhibitor in our system. Thus, plants were not receiving any benefit from the increase of fungal sporulation in these subplots. Because of all the unknowns with sporulation of *P. occultum*, considerable effort needs to be put into the patterns and plant responses to the sporulation of *P. occultum*.

Hypothesis 3: Mycorrhizal inoculum potential was significantly reduced by both fungicide and nitrogen application, leaving the subplot soil less able to colonize new host

plants (Fig. 2-9). Increases in sporulation in fungicide subplots (Fig. 2-4) did not lead to

increases in soil inoculum potential (Fig. 2-9). Interestingly, increases in inoculum

potential from 2006 to 2007 (Fig. 2-9), were patterned with increases in spore number

(Figs. 2-3, 2-4), and fungal richness (Fig. 2-7) from 2006 to 2007.

Hypothesis 4: Correlation analysis revealed that plant and fungal communities are

similarly impacted by fungicide and fertilizer application. Fungal richness was correlated

with plant productivity at the 1-m^{-2} scale (Fig. 2-12) as well as the 256-m^{-2} (subplot) scale

(data not shown). Correlation of fungal diversity with both plant diversity and

productivity has been observed by many authors (Hart and Klironomos 2002,

van der Heijden 2002, van der Heijden et al 1998a, 1998b, Kernahagan 2005).

Kernahagan (2005) concluded that the increase in plant productivity with increase in

diversity of mycorrhizal fungi likely occurs because of variation in fungal physiology in

such processes as nutrient uptake (Reynolds et al 2003, Vogelsang et al 2006), mycelial

growth and phosphorus foraging strategies (Jakobsen et al 1992, Smith et al 2000b).

Therefore, increasing variation in resource acquisition strategies with increasing fungal

diversity leads to increases in nutrient extraction efficiency (Kernahagan 2005).

In our system, it appears fungal diversity was not lost due to years of agricultural

production, and that fungal taxa were always present in the soil but growing at low,

undetectable levels. However, when provided suitable host plants, fungi quickly

rebounded, increasing root colonization and sporulation. Sporulation then continued to

increase as the plant community became more established. For our system, 3 years

apparently was a suitable time frame for reestablishment of a moderately-diverse fungal community.

Our results underscore the importance in maintaining fungal diversity within natural plant communities. The identity and diversity of fungal community can not only mediate plant diversity (Vogelsang et al 2006) but also can mediate specific plant-plant interactions (van der Heijden 2002, van der Heijden et al 1998a, 1998b). A plant's ability to access nutrients may depend on the presence of specific fungal taxa and in turn impacts survivability and fitness (van der Heijden et al 2003). While causation of plant-fungal interdependency cannot be determined from this study, it is clear that the two communities are reliant upon one another.

Like many previous studies, identifying the relationship between plant and fungal communities may be obvious, but determining the patterns and causation behind the observation is complex (Hart and Klironomos 2002, Read 2002). The difficulty lies in our lack of understanding and the obstacles in studying the underground fungal community (Read 2002). Much of the knowledge previous authors have gained in mycorrhizal relationships has come from focusing on the plant end of the relationship (van der Heijden and Sanders 2002, Read 2002). Thus, understanding the rules that guard plant community assembly requires a deeper knowledge of their mutualistic fungal partners and how specific fungal species interact with specific plant species.

Understanding of the importance of species composition of mycorrhizal fungal communities to restoration and assembly of plant communities is lacking. Many questions remain: Is there a threshold level of species, genera, or spore level, to project a

successful restoration or diverse plant community? Are there only a few important

keystone fungal taxa (Renker et al 2004) that need to reside within soil? What is known

is that mycorrhizal fungal communities are a strong factor that helps shape the assembly

and maintenance of natural plant communities.

CHAPTER III

INFLUENCE OF MYCORRHIZAL FUNGI

ON COMPETITION OF TWO PRAIRE GRASSES

ABSTRACT. Within their roots, many prairie plants harbor symbiotic fungi, called arbuscular mycorrhizal fungi (AMF), which assist plants in nutrient acquisition. Because plants respond differently to AMF, the fungi may influence how plants compete for resources. Two greenhouse studies were conducted to address how AMF affect the competitive interaction of two native warm-season grass species, big bluestem (*Andropogon gerardii* Vitm.) and Indiangrass (*Sorghastrum nutans* L.). Competition was assessed by growing individuals of the two species in pair-wise combinations of competitors (both inter- and intraspecific), and either 1) suppressing AMF, or 2) inoculating sterilized soil with AMF. Suppression of fungi altered the competitive balance between interspecifically-grown big bluestem and Indiangrass plants; however no evidence of mycorrhizal impact on intraspecific competition was observed among a species. AMF treatment altered root architecture of both, big bluestem and Indiangrass, even though they are closely related. Presence of mycorrhizal fungi increased total root length and total root biomass but lowered root branching patterns in both species. This research reinforces the importance of the below-ground component in influencing plant-plant interactions and, therefore, plant community assembly.

Introduction

It is well known that mycorrhizal fungi confer an advantage to individual plants, allowing the host greater tolerance to drought and soil borne disease, increased size, vigor, and yield (Allen 1991, Hetrick et al 1991, Smith and Read 2008). Because mycorrhizal symbiosis differentially impacts individual plant species by altering the resource acquisition ability of the plant, mycorrhizal fungi may also strongly influence plant-plant interactions (Hartnett and Fay 1998). These plant-plant interactions may have community level effects that determine plant diversity in a large area (Zobel and Moora 1995).

The relative benefit plants receive from the symbioses has a large impact on the competitive ability of the plant species involved (Moora and Zobel 1996). In nature, an individual plant competes with others for space, light, and nutrients. This competition is taking place between other plant species (interspecific competition) as well as with plants of the same species (intraspecific competition). An individual's ability to overcome competition of surrounding organisms facilitates survival and the opportunity to spread its genes throughout the population (Scheublin et al 2007). Any advantage an individual or population can gain over others may lead to greater reproductive success of the individual or population (Shumway and Koide 1995). Many studies have shown that mycorrhizal fungi can regulate interactions between competing plant species both intraspecifically (Ayers et al 2006, Facelli et al 1999, Marler et al 1999, Moora and Zobel 1996, Shumway and Koide 1995) and interspecifically (Fitter 1977, Hamel et al 1992, Hetrick et al 1989a, Moora and Zobel 1996, West 1996).

Several studies have shown mycorrhizal relationships increase the intensity of intraspecific competition (Ayers et al 2006, Watkinson and Freckleton 1997, West 1996). Under intense intraspecific competition, only a few individuals will be able to reproduce because of limitation of resources (Zobel and Moora 1997). Since mycorrhizal fungi may confer an advantage upon certain species relative to others, it is important to understand the role mycorrhizal fungi play in intraspecific competition in a wide range of plant species.

Most of the previous work performed on the interspecific competition has focused upon competing species that vary greatly in mycorrhizal (Hartnett et al 1993, Sanders and Koide 1994, Zobel and Moora 1997). However, only a limited number of studies exist involving plant species with a similar mycorrhizal response (Streitwolf-Engel et al 1997, West 1996).

Many studies have investigated how mycorrhizal fungi alter the competitive relationships between prairie plants; however these studies are often of plants in different functional guilds (C_3 and C_4 grasses) (Hetrick et al 1989a, Hartnett et al 1993, Wilson and Hartnett 1998). Without mycorrhizal symbiosis subordinate C_3 grasses (*Koeleria pyranidata*, *Elymus canadensis*) are able to out-compete the dominant C_4 prairie plant (*Andropogon gerardii*) (Hetrick et al 1989b, Hartnett et al 1993, Hartnett and Wilson 1999). However, in nature, these guilds are temporally separated with C_3 grasses growing in spring and fall and C_4 grasses growing during mid-summer; thus, competition for nutrients may be minimized. Little attention has been given to plants within the same functional guild, especially co-dominant, co-occurring warm season

prairie species. To understand plant community structure and assembly it is important to study interactions of common, closely related plants with similar responses to mycorrhizal fungi (Zobel and Moora 1997).

In the present study, mycorrhizal impact on plant competition was observed in an 8.1-ha (20-acre) experimental prairie established in Eau Claire County, Wisconsin. In fall 2003, the site was planted with combinations of native prairie species. The fungicide chlorothalonil was added to one-fourth of the study plots to suppress mycorrhizal fungi (and also other fungi). The fungicide addition resulted in a reduction of mycorrhizal root colonization by 35% (Stefanski et al in prep). After 3 years of treatment, the native grass big bluestem (*Andropogon gerardii*) was relatively abundant in untreated plots. However, in plots treated with fungicide, Indiangrass (*Sorghastrum nutans*) was more abundant (Weiher and Bentivenga, unpublished data). This led us to speculate that suppression of the mycorrhizal fungi altered the relative competitive ability of the two grasses, allowing Indiangrass to outcompete big bluestem. However, competition was not directly tested in the field experiment, so this remains speculation. The objective of the present study was to elucidate the mechanism behind the field observation. Based on these observations, it was hypothesized:

H_1: In this soil, big bluestem has a higher dependence upon mycorrhizal fungi than does Indiangrass.

H_2: Mycorrhizal fungi influence the competitive ability (both inter- and intraspecific) of two native prairie grasses (big bluestem and Indiangrass).

H_3: Observed competitive outcomes are driven by mycorrhizae and not soil-borne plant pathogens.

Materials and Methods

Experimental Design

To test these hypotheses, two separate greenhouse experiments were conducted. The first experiment addressed the competitive ability of big bluestem and Indiangrass in soil collected from the field site in Eau Claire County, Wisconsin, with chemically suppressed mycorrhizal fungi. Seed of Indiangrass and big bluestem were purchased from the same nursery that provided seed for the experimental prairie (Prairie Moon Nursery, Winona, Minnesota), to minimize plant genotype differences. Seeds were sterilized in 5% bleach for 20 minutes and allowed to dry. Seeds were stratified for 2 weeks in 1000 mg/L gibberellic acid in sterile flint sand, to increase germination rates (Watkinson and Pill 1998). Grasses were germinated in a sterilized flint sand / Turface (mixed 1:1) and then transferred to 6×25-cm "Deepots" (Stewe and Sons, Corvallis, Oregon), each containing approximately 600 g of untreated soil collected from the experimental prairie in Eau Claire. An estimate of the fungal spores present in each pot (based on sieving of field soil) is given in Table 3-1. Soil had a pH of 6.2 and contained 8 mg kg^{-1} of available P, 3.1-mg kg^{-1} of $NH_4 - N$, 6.4-mg kg^{-1} $NO_3 - N$, 54-mg kg^{-1} available K, 1,290-mg kg^{-1} available Ca, 336-mg kg^{-1} available Mg, and 2.6% organic matter as determined by the University of Wisconsin Soil and Plant Analysis Lab (Madison, Wisconsin).

Table 3-1: Estimated AMF spore communities per 600-ml pots in Experiments 1 and 2.

AMF species	Nonsterile soil (Exp 1)	Inoculum (Exp 2)
Glomus aggregatum	1590	365
Glomus etunicatum	1536	514
Glomus constrictum	1236	71
Paraglomus occultum	906	1036
Glomus intraradices	528	60
Glomus clavisporum	402	65
Scutellospora calospora	54	5
Scutellospora pellucida	48	10
Glomus mosseae	30	0
Gigaspora rosea	12	6
Gigaspora albida	12	0
Gigaspora gigantea	6	2
Gigaspora margarita	6	0
Entrophospora infrequens	6	0
Total	6372 Spores	2134 Spores

One-half of the pots were treated with the fungicide chlorothalonil at a rate of 0.08 mg per pot (equivalent to a field application of 24-mg m^{-2} yr^{-1}). Big bluestem and Indiangrass were grown in a pair-wise experimental design and were also both grown singly in pots. This allowed us to test both inter- and intraspecific plant competition (Fig. 3-1) (Gibson et al 1999, West 1996, Zobel and Moora 1995). Seedlings grown alone were centered in the pot, whereas the seedlings grown in pot pairs (BB:BB, BB:IG, IG:IG) were spaced approximately 2 cm apart. Each plant/fungicide combination was replicated eight times. Plants were arranged in the greenhouse in a completely randomized design (Cochran and Cox 1957). Plants were maintained at 15–25 C in the greenhouse for 12 weeks and were watered daily.
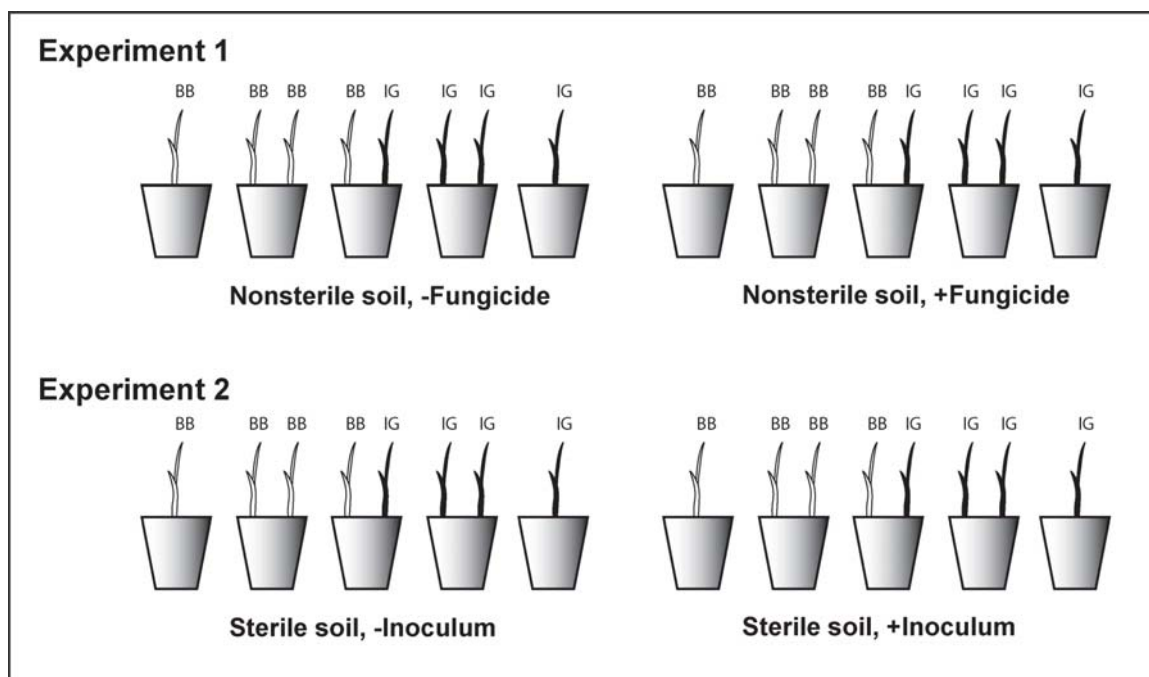
Figure 3-1. Pair-wise experimental design. BB = big bluestem, IG = Indiangrass

Plant height (from the crown to longest outstretched leaf) was measured weekly. At the 12th week, all above-ground plant material was harvested. Shoots for each plant were dried for 1 week at 50 C to determine above-ground biomass produced. Aboveground biomass was used to outcome of competition between target and competitor plant. The competitor could be either intraspecific (same species) or interspecific (opposite species) (*H2: Mycorrhizal fungi influence the competitive ability (both inter- and intraspecific) of two native prairie grasses (big bluestem and Indiangrass)*).

Wet below-ground biomass data were collected by washing soil away from root material for each pot. In two-plant pots (BB: BB, BB: IG, IG: IG), individual root

biomass could not be determined because the root systems were interwoven; therefore, below-ground biomass is reported as whole pot biomass.

To assess mycorrhizal colonization, a subsample (0.5 g wet biomass) of each root system was cleared and stained with trypan blue according to the method of Koske and Gemma (1989). Stained roots were examined microscopically for evidence of mycorrhizal colonization. The percentage of the root system occupied by mycorrhizal fungi was measured using the "grid-line intercept" method of Giovannetti and Mosse (1980). Mycorrhizal colonization data allowed us to determine mycorrhizal dependency of both species (H1: In this soil, big bluestem has a higher dependence upon mycorrhizal fungi than does Indiangrass.).

To test the third hypothesis (*H3: Observed competitive outcomes are driven by mycorrhizae and not soil-borne plant pathogens*), Experiment 2 was conducted. Experiment 2 had a similar experimental design to Experiment 1, however, soil treatment was altered. In this setup, collected field soil was steam-sterilized in a Hummert International 14 Soil Steamer for 2 hours at 90 C and then re-inoculated using 20 ml of inoculum from mycorrhizal fungal cultures (mixed species). Mycorrhizal fungi had been collected from the Eau Claire field site in the 2006 sampling year and had been maintained in the greenhouse, cultured on big bluestem and Sorgum-Sudangrass hybrid (var. Super Sv 22). Spores were collected from pot cultures by wet sieving, decanting and sucrose density centrifugation (Daniels and Skipper 1982). Spores were then suspended in deionized water, and 20 ml of the spore solution was pipetted onto the roots at the time of transplant. Spore density used as inoculum can be seen in Table 3-1. Spore

density was determined by enumerating a 20-ml random sample of the spore suspension at the time of inoculation. Control steamed pots received a 20-ml treatment of sieved (38 μm) suspension of non-sterilized soil to return other components of the natural soil micro-flora (*e.g.,*, bacteria), while still controlling AMF presence. Pasteurized soil was also sent to the University of Wisconsin Soil and Plant Lab (Madison, Wisconsin) for analysis. Steamed soil had a pH of 6.2, with 8-mg/kg of available P, 7.7-mg kg^{-1} of NH_4 –N, 3.5-mg kg^{-1} NO_3 –N, 42-mg kg^{-1} available K, 910-mg kg^{-1} available Ca, 384-mg kg^{-1} available Mg, and 2.4% organic matter. As in Experiment 1, plant height was measured weekly. Shoot and root dry weight, and root colonization were also measured after 12 weeks.

Statistical Analysis

Data from the two experiments were analyzed separately. Mycorrhizal dependency (H1) was determined by differences in above-ground biomass from single-grown individuals from Experiment 2. Mycorrhizal responsiveness was calculated as follows: [(inoculated biomass – uninoculated biomass)/inoculated biomass X 100 (Hetrick et al 1989b). To address Hypothesis 2 (competitive ability of grasses), above-ground biomass was analyzed for each species (big bluestem or Indiangrass) separately within the experiment (*e.g.,*, big bluestem in inoculated pots). Plant height and above-ground biomass data were highly correlated (r = 0.7890, p < 0.0001), thus only above-ground biomass data were used in further analyses. In the analysis, in interspecific pots (BB:IG) one species was designated as the target plant and one was held as the competitor; however, in the intraspecific pots (BB:BB; IG:IG) target plant and competitor

plants were chosen at random. Biomass for each species was compared according to its competitor species (alone, with big bluestem, or with Indiangrass) using a one-way analysis of variance (ANOVA) with SPSS, Version 16.0.

To determine differences in below-ground biomass, a 2-way ANOVA was performed using plant pairing (alone versus interspecific pairing versus intraspecific pairing) and soil treatment as the two main effects. To determine differences between 3 plant pairings, biomass data was subject to Tukey's HSD post hoc procedure.

Five root parameters (total biomass, root colonization, percent root length colonized, total root length, and total root length colonized) were also assessed. Because the roots of inter- and intraspecific pots could not be distinguished, root biomass was measured for the entire pot. After initial analysis in Experiment 1, no differences in the root parameters and fungicide and control plants were found and soil treatments were combined for Experiment 1 analysis. In Experiment 2, significant differences were found between inoculated plants and non-inoculated plants so; the 2-way ANOVA was used.

Root:shoot biomass ratios were determined in both single-grown pots as well as in the paired pots. In paired pots, above-ground biomass of both plant species were combined to determine the overall pot root:shoot ratio. Interspecific root:shoot biomass was done similarly as well because below-ground biomass values were statistically indistinguishable between big bluestem and Indiangrass when grown alone.

Results

Mycorrhizal response -- As expected, both species showed a high responsiveness to mycorrhizal fungi. Indiangrass showed 90% responsiveness, and big bluestem showed 82% responsiveness.

Experiment 1

Root Colonization -- Because no significant differences were seen in colonization rates among the soil treatments (fungicide application) (Fig. 3-2), soil treatments were combined for the analysis of Experiment 1. Although no differences were observed among the soil treatments in root colonization, differences were observed between the plant species (Fig. 3-3a, BB-0 vs IG-0). Big bluestem had much lower mycorrhizal colonization than did Indiangrass, with rates of 11% and 25% respectively.
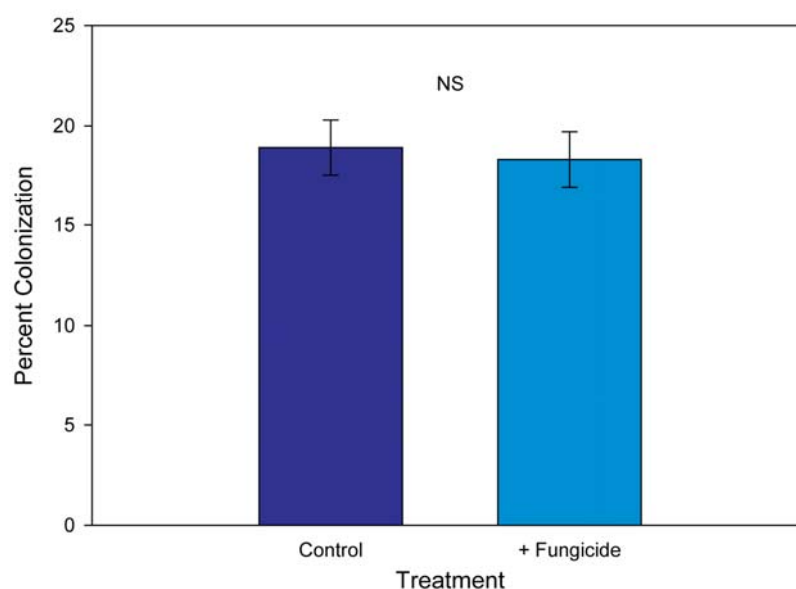
Figure 3-2. Root colonization in Experiment 1 with plant pairings combined. Error bars represent 95% confidence intervals.

Experiment 1 (Non-sterile field soil)

Experiment 2 (Sterile field soil)
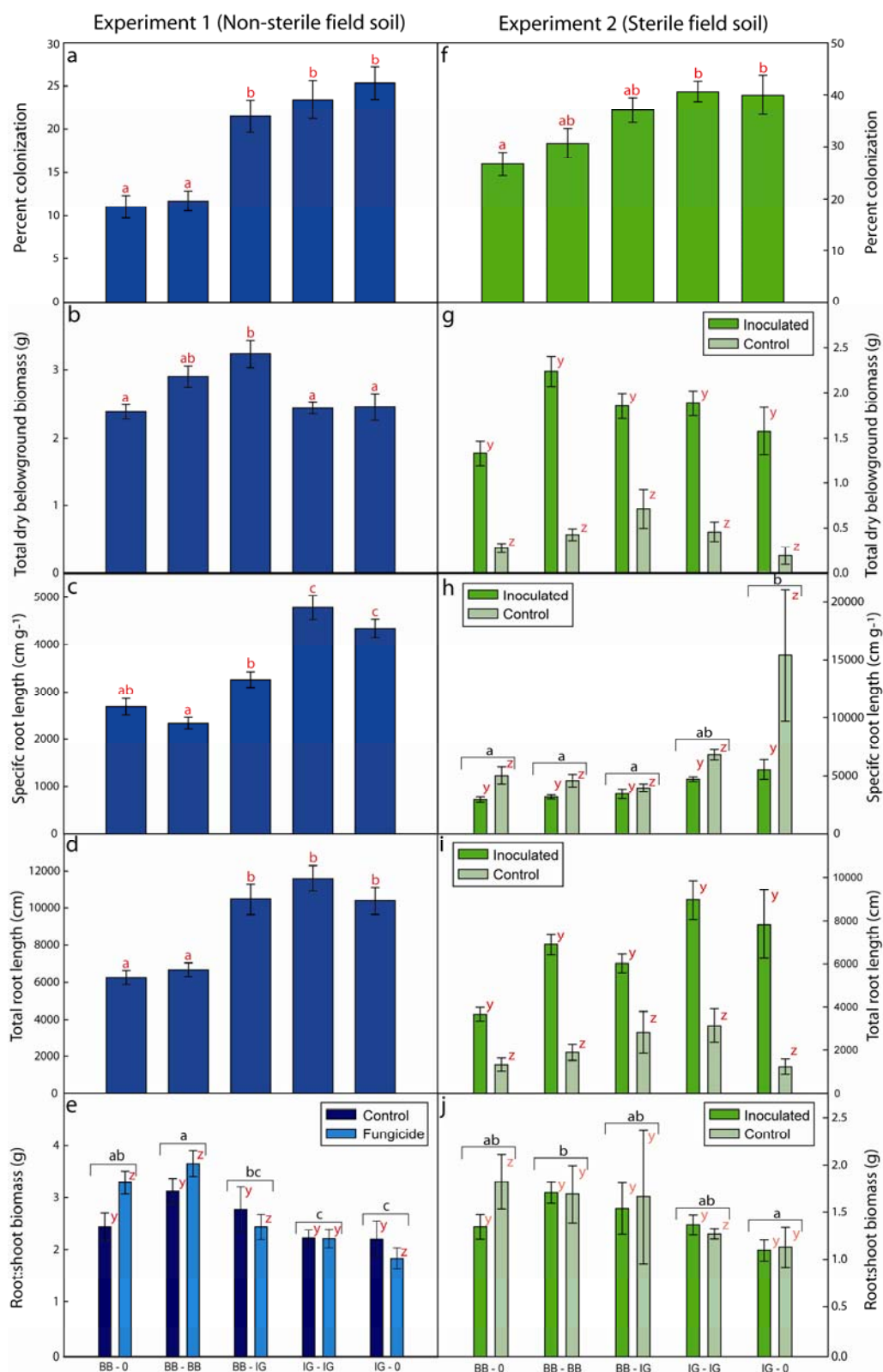
Figure 3-3. Measured root parameters for Experiment 1 and 2. a) Mean percent

colonization by AMF among five plant pairings in non-sterile soil. Letters (a, b, c) for all

panels indicate significant differences between pairings (treatments combined), according

to Tukey's HSD procedure. Error bars represent 95% confidence intervals. b) Total dry

below-ground biomass in non-sterile soil. c) Specific root length (cm g^{-1}) in non-sterile

soil. d) Estimated total root length (cm) in non-sterile soil. e) Root:shoot biomass ratio

in non-sterile soil. Within each pairing, means with letters (y, z) differ significantly,

according to ANOVA. f) Mean percent colonization by AMF in sterile soil. g) Total dry

below-ground biomass in sterile soil. h) Specific root length (cm g^{-1}) in sterile soil. d)

Estimated total root length (cm) in sterile soil. e) Root:shoot biomass ratio in sterile soil.

Above-ground biomass production -- Not surprisingly, biomass production was greatest for both species when grown alone (Figs. 3-4 ●, 3-5 ●). Within the single-grown big bluestem plants (Fig. 3-4 ●), biomass did not change with or without fungicide application and mean above-ground biomass was about 0.8 g. Single-grown Indiangrass above ground biomass also did not change when given fungicide and was about 1.3 g in both treatments (Fig. 3-5 ●).
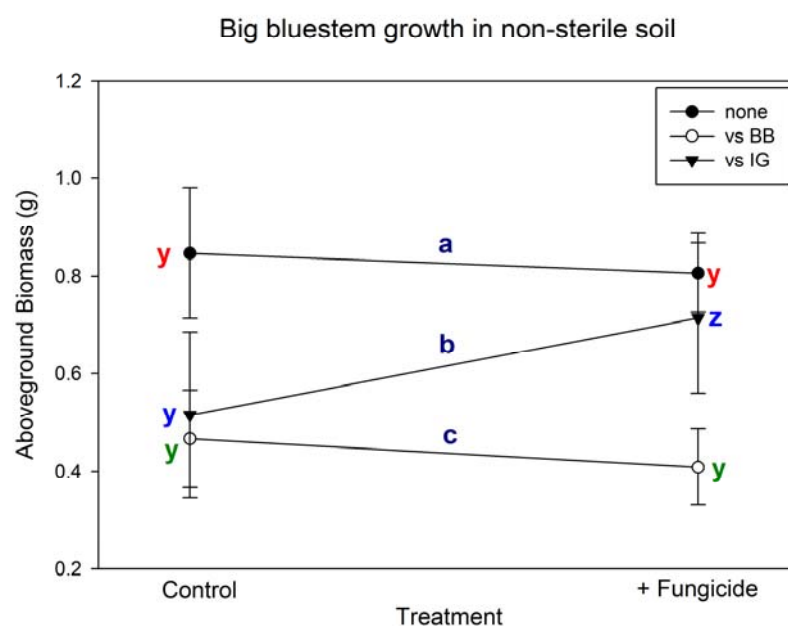
Figure 3-4. Above-ground biomass of big bluestem grown alone, with big bluestem or with Indiangrass in non-sterile soil. Within each competitor, means with y, z differ significantly, according to ANOVA. Letters attached to lines (a, b, c) indicate significant differences between competitors (treatments combined), according to Tukey's HSD procedure. Error bars represent 95% confidence intervals.

Indiangrass growth in non-sterile soil
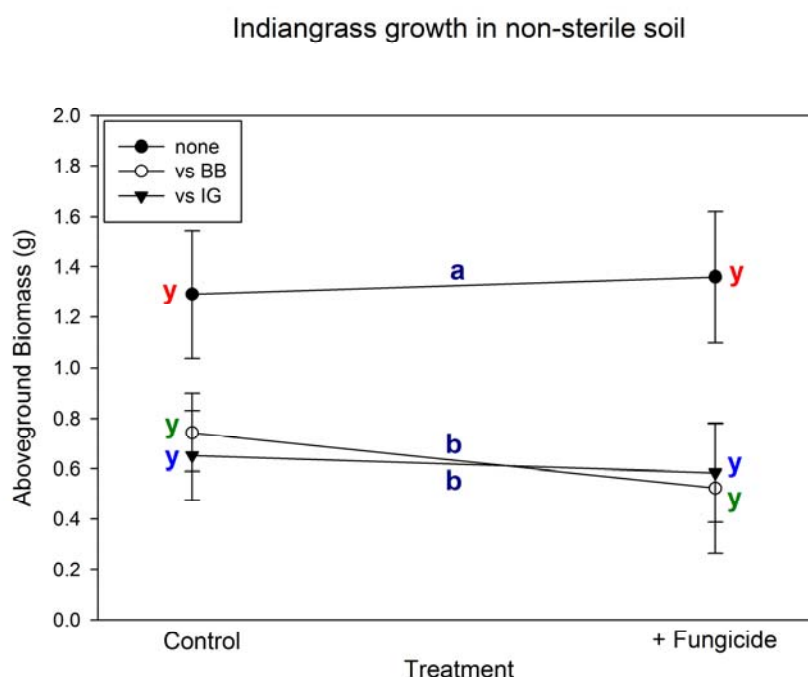
Figure 3-5. Above-ground biomass of Indiangrass grown alone, with big bluestem or with Indiangrass in non-sterile soil. Letters (a, b) indicate significant differences between competitors (treatments combined), according to Tukey's HSD procedure. Error bars represent 95% confidence intervals.

Above-ground biomass of intraspecific big bluestem and Indiangrass was about half when compared to biomass production in single-grown pots (Figs. 3-4 ○, 3-5 ▼). Target big bluestem and Indiangrass biomass when grown intraspecifically did not change when given fungicide addition (Figs. 3-4 ○, 3-5 ▼).

Interspecific growth of target big bluestem was altered by addition of fungicide (Figs. 3-4 ▼, 3-5 ○). Growth of Big bluestem control (Fig 3-4 ▼) was about 0.5 g (about half of single-grown big bluestem ●), whereas biomass of big bluestem with fungicide

addition was similar to single-grown big bluestem plants (about 0.8 g) (Fig. 3-4 ▼, ●).

Interspecific growth of target Indiangrass was not altered by fungicide addition

(Fig. 3-5 ○). Biomass production of interspecific Indiangrass (Fig. 3-5 ○) was about

0.7 g (half the biomass of single-grown Indiangrass (Fig. 3-5 ●)).

In a comparison of interspecific pots side by side (Fig. 3-6), in control pots,

Indiangrass (○) produced more biomass than did big bluestem; however, in fungicide

pots, big bluestem (●) produced more biomass than did Indiangrass.
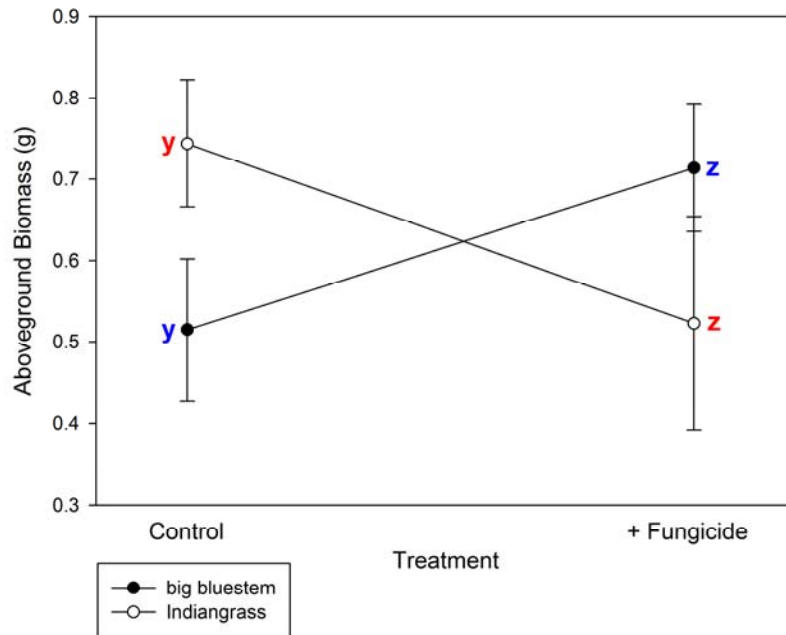
Figure 3-6. Above-ground biomass of Interspecific pots (big bluestem versus

Indiangrass) in non-sterile soil (Exp 1). Within each competitor, means with y, z differ

significantly, according to ANOVA. Error bars represent 95% confidence intervals.

Root Parameters -- Below-ground biomass production in big bluestem was similar with and without fungicide addition; for simplicity, data for treatments were pooled in Fig. 3-3b. Big bluestem plants produced 2.5 g of total below-ground biomass in single-grown pots, which was equal to the amount of biomass produced by single-grown Indiangrass (Fig. 3-3b). Intraspecific big bluestem and Indiangrass biomass were similar to levels observed in single-grown big bluestem and Indiangrass pots (Fig.3-3b). Below-ground biomass in Interspecific pots was significantly higher than all other pots, with 3.2-g of biomass produced (Fig. 3-3b).

Although plants produced similar amounts of below-ground biomass, plants differed greatly in overall root architecture. Comparison of total root length between single-grown big bluestem and Indiangrass showed that Indiangrass producing nearly double the root length of big bluestem with values of 10 m and 6 m, respectively (Fig. 3-3d). Intraspecific-grown big bluestem had similar root length as single-grown big bluestem, with 6 m of root length produced (Fig. 3-3d). Intraspecific Indiangrass produced 11 m of root length, which was similar to single-grown Indiangrass (Fig.3-3d). Interspecific pots produced 10 m of root length, which was similar to single-grown Indiangrass (Fig. 3-3d).

Because both species produced a similar amount of biomass, single-grown Indiangrass had double the specific root length, which is a determinant of centimeters of root length per gram of root material (Fig. 3-3c). Thus, Indiangrass produced smaller diameter, finer root systems than did big bluestem.

Fungicide addition had a significant impact on biomass allocation of these two grasses, significantly altering root:shoot ratios (Fig. 3-3e). However, fungicide did not have a similar impact across pot pairings. In single-grown big bluestem species, fungicide application led to an increase in root:shoot ratios, with 2.5 g of root to 1 g shoot in control pots to a 3.2 g of root to 1 g shoot in fungicide pots (Fig. 3-3e). Alternatively, fungicide application within single-grown Indiangrass plants led to a reduction in root:shoot ratios (Fig. 3-3e). In control pots, single-grown Indiangrass produced 2.1 g of root to 1 g shoot, which was reduced to < 2 g of root to 1 g shoot with fungicide application (Fig. 3-3e).

Root:shoot ratios in intraspecific big bluestem increased with fungicide application, similar to single-grown big bluestem (Fig. 3-3e). Application of fungicide had no impact on the root:shoot ratio of intraspecific Indiangrass (Fig. 3-3e). Fungicide application reduced root:shoot ratios in Interspecific pots from 2.8 g of root per 1 g shoot in control pots to 2.5 g of root per 1-g shoot.

<div align="center">

Experiment 2

</div>

Root Colonization -- Steam pasteurization adequately sterilized field soil. Examination of non-inoculated plant roots revealed no mycorrhizal colonization (data not shown). Within inoculated plants, Indiangrass had increased levels of colonization when compared to big bluestem, with levels of about 40% in Indiangrass and 28% in big bluestem (Fig. 3-3f, BB-0, IG-0).

Above-ground biomass production -- In sterile soil, both big bluestem and Indiangrass

benefited significantly from mycorrhizal colonization (Figs. 3-7 ●, 3-8 ●). In sterile soil,

non-inoculated plants of both species were highly stunted (Figs. 3-7, 3-8). Inoculation of

single-grown big bluestem plants led to a 5-fold increase in biomass production, from

0.2 g to 1.0 g (Fig. 3-7 ●). Single-grown Indiangrass displayed a 3-fold biomass increase

when inoculated (Fig. 3-8 ●).
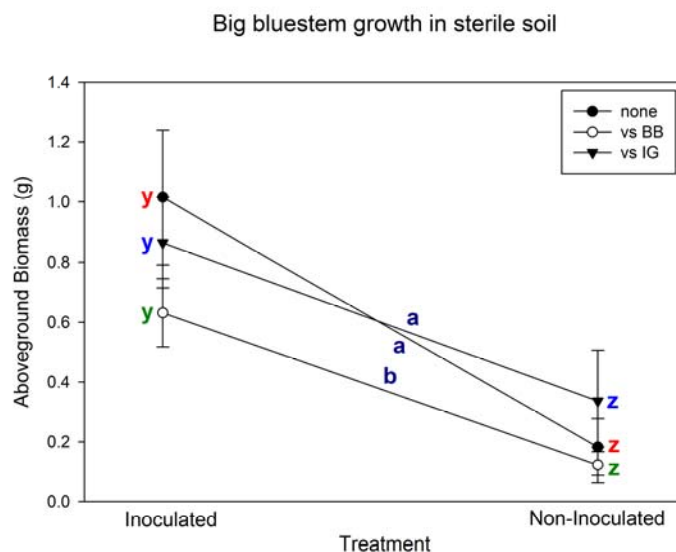
Figure 3-7. Above-ground biomass of big bluestem grown alone, with big bluestem and

with Indiangrass in sterile soil. Within each competitor, means with y, z letters differ

significantly, according to ANOVA. Letters (a, b) indicate significant differences

between competitors (treatments combined), according to Tukey's HSD procedure. Error

bars represent 95% confidence intervals.
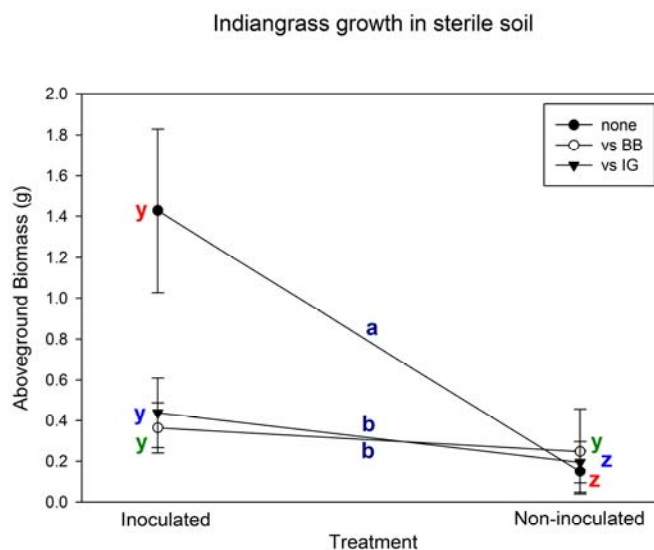
Figure 3-8. Above-ground biomass of Indiangrass grown alone, with big bluestem and with Indiangrass in sterile soil. Within each competitor, means with y, z letters differ significantly, according to ANOVA. Letters (a, b) indicate significant differences between competitors (treatments combined), according to Tukey's HSD procedure. Error bars represent 95% confidence intervals.

Inoculated intraspecific big bluestem plants achieved 3-fold growth over control plants from a mean of 0.2 g biomass to 0.6 g biomass with inoculation (Fig. 3-7 ○). Inoculation of intraspecific Indiangrass plants doubled in biomass from 0.2 g to 0.5 g when compared to control plants (Fig. 3-8 ▼).

Inoculation of target big bluestem in interspecific pots led to a 2.5-fold biomass increase compared to control pots (Fig. 3-7 ▼). However, inoculation of target Indiangrass in intraspecific pots did not promote any biomass increases when compared

to control pots (Fig. 3-8 ○). Comparison of interspecific pots with big bluestem

(Fig. 3-9 ●) and Indiangrass (Fig. 3-9 ○) side by side reveals that inoculation allows big

bluestem to achieve 2.5x the biomass of Indiangrass in the same pot. However, in control

pot, biomass production was equal among the two species (Fig. 3-9).
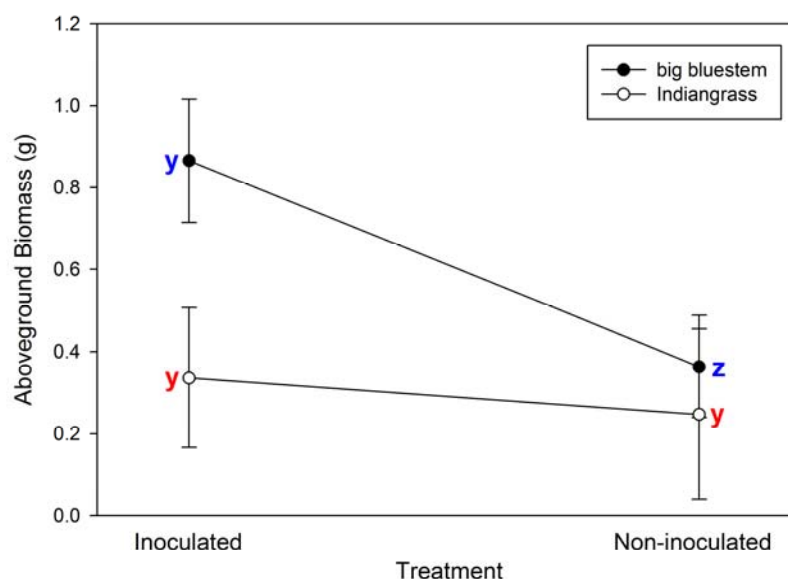
Figure 3-9. Above-ground biomass of interspecific pots (big bluestem versus

Indiangrass) grown in sterile soil (Exp 2). Within each competitor, means with y, z

letters differ significantly, according to ANOVA. Error bars represent 95% confidence

intervals.

Root Parameters -- Below-ground biomass production of single-grown big bluestem

increased five-fold from 0.3 g to 1.4 g with inoculation (Fig. 3-3g). A similar trend was

observed in single-grown Indiangrass, which had a mean below-ground biomass of

1.6 g with inoculation (Fig. 3-3g). Growth of intraspecific big bluestem and Indiangrass both achieved a 4-fold increase in biomass production with inoculation (Fig. 3-3g)

Uninoculated interspecific pots had much higher biomass production than any other uninoculated planting treatment (Fig. 3-3g). Inoculation allowed a significant increase in biomass in the intraspecific pots, raising biomass from 0.7 g in control pots to 2.0 g in inoculated pots (Fig. 3-3g).

Observations made in total root length were similar to observations made in below-ground biomass (Fig. 3-3i). All planting mixtures received considerable benefit from inoculation and were able to significantly increase total root length (Fig. 3-3i). However, inoculation of plants allowed all planting mixtures to lower specific root length. Single-grown big bluestem that received inoculation had specific root lengths that were half of control big bluestem plants (Fig. 3-3h). Inoculation of single-grown Indiangrass plants reduced specific root length by 66% when compared to control plants (Fig. 3-3h). Intraspecific big bluestem and Indiangrass pots and interspecific pots all lowered specific root length when inoculated (Fig. 3-3h).

Single-grown big bluestem lowered root:shoot ratios from 1.9-g to 1.4-g (Fig. 3-3j). Root:shoot ratio of single-grown Indiangrass was not impacted by inoculation and remained 1.2 g (Fig. 3-3j).

Root:shoot ratio of intraspecific big bluestem pots was not influenced by fungal inoculation (Fig. 3-3j). Inoculation of intraspecific Indiangrass pots led to a rise in root:shoot ratios from 1.30 g to 1.45 g (Fig. 3-3j). Root:shoot ratios of interspecific pots did not significantly differ due to inoculation when compared to controls (Fig. 3-3j).

Discussion

Mycorrhizal Response

Big bluestem and Indiangrass were both highly responsive towards mycorrhizal fungi. Many authors have previously described the strong association of these two warm-season grasses with mycorrhizal fungi in prairie soils (Hartnett et al 1993; Hetrick et al 1989a, 1991, 1994; Wilson and Hartnett 1997, 1998) In Eau Claire soil, Indiangrass displayed higher responsiveness to mycorrhizal fungi than did big bluestem, with values of 90% and 82% respectively. Data do not support the first hypothesis that big bluestem has a higher dependency on mycorrhizal fungi than does Indiangrass, and thus, it is rejected.

Root Colonization and Above-ground Competition

Surprisingly, addition of fungicide did not lead to decreases in colonization rates (Fig 3-2), which led us to believe we did not add sufficient fungicide to adequately suppress mycorrhizal activity. Upon analysis of fungicide application rates given to greenhouse pots compared to field application rates, pot application far undershot field application. Our application rate to greenhouse pots was calculated for a field rate of 24-mg m^{-2} yr^{-1}, while field application was 15g-m^{-2} yr^{-1}. This led us to believe the initial concentration of fungicide was insufficient to suppress mycorrhizal fungi. Fungicide application was lowered from field to greenhouse because of toxicity symptoms observed within the field as well as greenhouse plants being pot bound; there were no soil runoff issues. In field work conducted at the Eau Claire prairie field site by Stefanski et al (in preparation), mycorrhizal root colonization was decreased by 35% in plots with fungicide

application. Despite the lack of reduction in colonization, fungicide application had a measureable impact on the competitive abilities of big bluestem and Indiangrass. Application of fungicide led to a decrease in Indiangrass above-ground biomass and an increase in big bluestem above-ground biomass.

Because no reduction in single-grown Indiangrass or big bluestem above-ground biomass production occurred (Figs. 3-4 ●, 3-5 ●), it was unlikely that any of the plants showed toxic effects. One possibility for the decrease in growth of big bluestem was that an unidentified big bluestem-specific pathogen resided within nonsterile field soil. It was possible that fungicide application was adequate to suppress this pathogen but not sufficient to suppress mycorrhizal fungi. Suppression of the pathogen would release big bluestem roots from this interaction and allow the increase in biomass, which would come at the expense of Indiangrass. However, there was no evidence supporting big bluestem pathogen upon examination of root material. Another possibility was that our fungicide did suppress some components (species) within the pots, but other members of the AMF community were able to survive. However, since no DNA sequencing of root material was performed, this is mere speculation.

In Experiment 2, above-ground biomass of big bluestem in intraspecific pots was statistically equal to what it was alone with inoculation (Fig. 3-7 ○). Growth of intraspecific Indiangrass was similar with or without fungal inoculation (Fig. 3-8 ▼). This indicates that Indiangrass was minimally able to impact the growth of big bluestem in this system or that these two species under these greenhouse conditions are amensal. In the field, big bluestem had higher abundance levels in control plots than did

Indiangrass, and the opposite was observed in plots with fungicide application, with Indiangrass having higher abundance levels than big bluestem.

No evidence of mycorrhizal fungi impacting intraspecific competition was observed; thus, fungicide application influenced interspecific but not intraspecific competition, supporting hypothesis 2. However, no evidence exists that mycorrhizal fungi were suppressed by fungicide application, leading us to reject the third hypothesis, that competition is being driven by mycorrhizal fungi and not soil-borne pathogens.

Root Parameters

Indiangrass and big bluestem are closely related in physiology, anatomy, phenology, ecology, and both are warm-season C_4 grasses. However, they differed in rooting strategy. When grown singly or intraspecifically, both species produced a similar level of biomass underground (Fig. 3-3b). However, Indiangrass produced much longer and finer roots (Figs. 3-3c, d). The specific root length was higher in Indiangrass, indicating a much finer root system when compared to big bluestem (Fig. 3-3c). Because both plants are highly dependent on mycorrhizal fungi, showing response levels of 90% for Indiangrass and 82% for big bluestem, it was unexpected to find these differences in rooting architecture. Many authors have found that plants with a higher responsiveness to mycorrhizal fungi typically have a less branched, coarser rooting system when compared to plants with a lower responsiveness to mycorrhizal fungi (Hetrick et al 1991, Schultz et al 2001). Surprisingly, greater responsiveness to mycorrhizal fungi was observed in Indiangrass, which had a finer, more branched rooting structure. Although Indiangrass had a finer rooting system than big bluestem, specific root lengths were still

much lower than the specific root lengths of grasses that showed little responsiveness to mycorrhizal fungi (i.e. C_3 grasses).

Although the results were not as expected, insight was gained. The differences observed in rooting architecture provide a possible explanation for the competitive differences in these grasses in the greenhouse study as well as the manipulated prairie field site. Because Indiangrass has a higher root branching system, this may give Indiangrass an advantage in fungicide subplots. Big bluestem may be dependent upon the mycorrhizal fungal community associated with the control subplots. However, this is only speculation, and more work needs to be conducted on the interaction of these co-occurring grass species within the prairie system.

Our results also show that the mechanisms controlling plant interactions are complex. Though the soil and fungal community used for both experiments was collected from the same field site, growth of the plant pairings differed between experiments. Because these plants showed variation in response to mycorrhizal fungi between Experiment 1 and Experiment 2, comparison of the two experiments is not valid.

To determine the impact of mycorrhizal fungi on inter- and intraspecific competition in both greenhouse and in the field, more work needs to be conducted in which usage of differing competition setups are tested, which take into account variation in plant densities, phosphorus gradients, and fungicide gradients. Once patterns could be established in the greenhouse, competitive experiments should be performed in the field. To determine the outcome of competition, multi-generational studies could be carried out to investigate if patterns observed in the greenhouse lead to changes in the abundance

levels of competing plant species in the field. An understanding of how mycorrhizal fungi can impact the competitive outcomes of plants within prairie communities could shed some light into how prairie plant communities assemble themselves.

CONCLUSIONS

Studies conducted as part of this thesis underscore the importance and complexity of the rules governing plant community assembly and maintenance. I have found that soil factors such as microbes and nutrition can impact both assembly and maintenance of plant communities. Mycorrhizal fungi play a key role in influencing plant productivity, nutrition, competition, and therefore survivability, and also influence soil structure and stability. Because mycorrhizal fungi directly and indirectly impact these above factors, mycorrhizal fungi may play an important role in ecosystem functioning. Because mycorrhizal fungi have the ability to alter plant community structure (van der Heijden et al 1998), conservation and restoration of vital habitats such as the North American tallgrass prairie must be accompanied by an active maintenance and restoration of the mycorrhizal community. This is especially important to restoration of areas that have a long agricultural history. It has been demonstrated that many common agricultural practices (fertilization, monoculture, tillage) have negative impacts on the mycorrhizal community. In turn, alterations of the fungal community are mirrored by the abundance patterns of the plant community (Chapter II). Decreasing fungal diversity lowers plant diversity and productivity and alters patterns of plant species co-existence both in the field (Chapter II) and in the greenhouse (Chapter III).

Active maintenance of fungal diversity may be the missing link in restoration of lost habitats. Because of fungal-plant preferences, occurrence and abundance of certain plant species in a community may be linked to the presence of certain mycorrhizal fungal

species and *vice versa* (Read 1998, Renker et al 2004). Therefore, it is likely that the overall benefit to the plant community (increases in diversity, biomass, productivity, and density) intensifies with increased fungal diversity (van der Hiejden 2003).

Restoration ecology is not a simple science; to adequately restore natural habitats, such as prairies, requires a multi-layered approach in which plant seeding mixture is only one of the layers. Though restoration ecologists have initiated the usage of mycorrhizal fungi as a restoration tool, the role of fungi in this capacity remains largely unexamined and underutilized (Hart and Klironomos 2002). One reason for this may be the lack of knowledge and understanding of this underground community. Hopefully, this thesis can be used to shed some light on this unseen community.

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