LIMITATIONS TO PLANT DIVERSITY AND PRODUCTIVITY IN RESTORED TALLGRASS PRAIRIE

by

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B.A., Augustana College, 2002
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Abstract

Approximately 96% of native tallgrass prairie in North America has been lost, which accentuates the need for effective methods to restore the structure and function of these degraded ecosystems. Many prairie restorations aim to restore grass and forb species in proportions reflecting plant species diversity in native prairie. A target grass-forb species mixture is typically chosen at the onset of restoration, but often, grasses become excessively dominant and forbs are underrepresented as the community develops. Several studies have examined the potential for increasing forb cover and diversity in newly restored grasslands, but few studies have assessed factors limiting forb cover and diversity in well-established grass-dominated prairie restorations. The primary objective of this research was to assess the potential for enhancing plant species diversity and productivity in an established grass-dominated prairie restoration by selective removals of dominant grass species, and by manipulating resources (soil nutrients, light availability) or mycorrhizal interactions.

A 7-year old grass-dominated restoration was used to evaluate plant and soil responses to manipulations in three separate studies. The first study examined the potential suppressive effects of dominant grasses on plant diversity by reducing the cover and biomass of two dominant grass species, *Andropogon gerardii* and *Panicum virgatum*. After 3 years, the removal of *A. gerardii* increased species richness and diversity, which was correlated with increased light availability, but not changes in soil resources. The second study examined the responses of restored grassland communities to long-term manipulation of soil resources (nutrient availability or soil depth), and to aboveground biomass removal via mowing. The long-term manipulation of soil resources did not alter plant species diversity, but nitrogen and light availability were important factors regulating plant productivity. The third study assessed the effects of manipulating arbuscular mycorrhizal (AM) fungi, through the use of either commercial inoculum or fungicide, on plant communities in restored prairie. Mycorrhizal suppression reduced grass productivity, suggesting that fungicide may be useful for enhancing diversity of restored prairies that are dominated by obligate mycotrophic grasses. In total, these studies suggest that competition between dominant grasses and subordinate forbs limits plant diversity in restored tallgrass prairie.

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CHAPTER 1 - INTRODUCTION

What is restoration ecology and ecological restoration?

Restoration ecology is a science that provides practitioners with concepts, models, methodologies and tools to support the practice of ecological restoration (SER 2004). Restoration ecology has also been described as the "scientific process of developing theory to guide restoration and using restoration to advance ecology" (Palmer et al. 2006). Ecological restoration has been defined as "the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed", and is the "intentional activity that initiates or accelerates the recovery of an ecosystem with respect to its health, integrity and sustainability" (SER 2004). Even though these are the definitions provided by the Society for Ecological Restoration, the use of the words "health", "integrity", and "sustainability" in the context of restoration are value-laden and fraught with ambiguity. Palmer and others (2006) provide a somewhat more objective definition of ecological restoration as the "attempt to recover a natural range of ecosystem composition, structure, and dynamics" to a degraded site (Palmer et al. 2006), although it can also be difficult to determine what constitutes a "natural range". Because ecosystems in need of restoration are often conceived as operating outside the bounds of some nominal range of community composition or ecosystem processes, an appropriate goal for restoration might be "to move a damaged system to an ecological state that is within some acceptable limits relative to a less disturbed system" (Palmer et al. 2006). Again, defining what constitutes "acceptable limits" and "less disturbed" is often difficult and subjective.

Ecological restoration includes a range of management practices, such as reforestation, habitat and range improvement, and erosion control, just to name a few. Some forms of ecological restoration are hundreds or even thousands of years old (Anderson 2005), but ecological restoration of tallgrass prairie ecosystems, on the other hand, is a relatively young practice. The first ecological restoration of a prairie ecosystem is generally attributed to a small prairie restoration at the University of Wisconsin Arboretum by Aldo Leopold in the fall of 1935 (Jordan et al. 1987; Perrow and Davy 2002). Even though various forms of ecological restoration have been practiced for a long time, the science of restoration ecology as an academic field has only emerged in the last two decades. With this emergence, there is an increasing need

to define the relationship between ecological restoration and the science of restoration ecology (Young et al. 2005).

Traditionally, ecological restoration has been based on trial and error and a site-based perspective, in particular restoring well-defined areas using methods tailored to a specific site (Hobbs 2002). The central goal of most restorations is to promote autogenic processes that aid in the recovery of ecosystem structure and function until the ecosystem can function with acceptable limits without further assistance from the restorationist, thereby becoming self-sustaining (SER 2004). In general, a restoration plan includes a clear rationale for why the restoration is needed, clear goals and objectives for the restoration, an explanation of how the restoration will integrate with the existing flows of the landscape, and strategies for long-term protection and maintenance until self-sustainability is met (SER 2004). Hobbs (1999) outlines four reasons that restorations may be needed, which include the following: (1) to restore highly disturbed localized sites, (2) to improve production in degraded or damaged agricultural, range, and forest lands, (3) to enhance conservation values in protected areas, and (4) to restore ecosystem structure and function over landscape-scales or regional areas.

Restoration success is based on reintroducing "valued processes" with sufficient biotic and abiotic components to allow an ecosystem to continue to develop without further assistance by practitioners (SER 2004). In other words, a successfully restored ecosystem should be resilient and able to recover after stresses typical of that particular ecosystem (e.g., drought in arid grasslands, fire or grazing in mesic grasslands) (Palmer et al. 2006). However, success can be both difficult to measure and difficult to achieve (Allison 2002; Anand and Desrochers 2004; Martin et al. 2005), and in some cases long-term management of restored ecosystems may be required (Palmer et al. 2006). Restoration also can be used as an 'acid test' for ecology (Bradshaw 1987), which means restoration ecology can be used to test ecological knowledge and theory. With this new relationship between basic ecological principles and restoration, the field of restoration ecology has expanded, and the practice of ecological restoration has become one of the most extensive and expensive conservation actions around the world (Holl et al. 2003). In summary, restoration ecology provides a unique opportunity to test ecological theory, and in turn, ecological theory can be useful in guiding restoration.

Why is restoration ecology needed in tallgrass prairie areas?

Humans require goods and services from natural ecosystems, but at the same time have the ability to induce environmental change that impacts these same goods and services. As the human population continues to grow, the demand for ecosystem goods and services will also increase resulting in continued degradation and added stress to already weakened ecosystems, such as the tallgrass prairie. With these added demands, conservation or simple maintenance of the current ecosystems will not be enough (Hilderbrand et al. 2005). Human consumption and demand will need to change, or a shift to creating, restoring, and enhancing ecosystems and their services will need to occur at a greater rate (Hilderbrand et al. 2005). A proactive, versus reactive, approach is necessary to protect, conserve, and restore the multi-functionality of ecosystems; otherwise, the remnants of these systems will continue down the degradation path without the hope of future rejuvenation.

Prior to European settlement, the North American tallgrass prairie covered more than 68,000,000 ha of the Great Plains (Samson and Knopf 1994; Robertson et al. 1997), but since European settlement more than 96% of the tallgrass prairie has been lost due to conversion to agriculture, fragmentation, exotic species invasion and fire suppression (Samson and Knopf 1994). With this loss, prairie ecosystems have experienced extensive alterations in ecosystem processes and community composition resulting in overall degradation. The degradation of the tallgrass prairie has led to decreased biodiversity and ecosystem services, and increased risk of invasibility by exotic species (Webb 1996; Hoekstra et al. 2005). The decline of prairie ecosystems accentuates the need to develop restoration methods to restore community composition and ecosystem services (Webb 1996).

What are the theoretical foundations of restoration ecology?

Restoration ecology aims to rebuild functioning ecosystems, and a community or ecosystem-level perspective is necessary to achieve this. Restoration ecology incorporates several different ecological theories and concepts including ecological genetics, ecophysiology, demography, community ecology, evolutionary ecology, food webs, biodiversity, macroecology, ecosystem ecology, and paleoecology (Palmer et al. 2006), with the issues and themes from community ecology being most relevant to the chapters that follow. Community ecology encompasses a wide variety of issues and themes including, but not limited to, species

coexistence and interactions, disturbance, and successional trajectories. These issues are important during ecological restoration since community interactions (above- and belowground) influence the structure and dynamics of restored plant communities.

Biotic interactions must be considered during restoration since organisms do not live in isolation and these interactions may influence the outcome of the restoration (Menniger and Palmer 2006). Competition for shared resources (above- and belowground) is important to consider during restoration because interactions between and within species may prevent species from establishing in a community (Menniger and Palmer 2006), or alter trajectories of change in communities. In prairie restorations, competition theory is important because warm-season grasses, such as big bluestem (*Andropogon gerardii*), often become dominant and may out-compete subordinate grass and forb species for light, space or nutrients or any combination of these resources (Menniger and Palmer 2006). Identifying how competition affects a restoration specifically may be difficult, but restoration ecology provides a framework to test how competition may limit community recovery in a restoration.

Besides competition for shared resources, mutualism is another important biotic interaction influencing restored plant communities. Arbuscular mycorrhizal (AM) fungi are ubiquitous in all plant communities, with approximately 80% of vascular plant species forming this association (Harley 1971). The beneficial relationship between AM fungi and host plants is well documented. Plants benefit by having increased nutrient uptake, increased drought tolerance, and protection from root pathogens (Perrin 1990; Fitter 1991; Marschner and Dell 1994; Ruiz-Lozano and Azcon 1995). In return, the plants allocate as much as 26% of the carbon fixed by photosynthesis to the fungal symbiont (Rillig 2004; van der Heijden et al. 2006). AM fungi can play a role in restoration, particularly in sites that are degraded in ways that negatively impact spore counts and infectivity of mycorrhizal fungi (Moorman and Reeves 1979; Jasper et al. 1989; Smith et al. 1998). Manipulating AM fungi during restoration may enhance restoration efforts since AM fungi positively influence the aboveground plant community (Noyd et al. 1996; Thorne et al. 1998; Smith et al. 1998), and reestablishing AM fungi in severely degraded ecosystems "may be very important to the outcome of a restoration project" (Menniger and Palmer 2006).

Natural disturbances (e.g. fire, floods, etc.) are significant in shaping community structure, especially in prairies, forests, and rivers (Sousa 1984). Disturbance is a source of

organism mortality and displacement, but it also provides a source of environmental heterogeneity and influences key ecosystem processes (Menniger and Palmer 2006). Humans have altered many natural disturbance regimes, and in many restorations incorporation of a natural disturbance regime, or one that mimics natural disturbances, may be needed for the recovery of ecosystem processes. For instance, in native tallgrass prairie ecosystems, fire and grazing are important factors that shape plant community structure (Abrams and Hulbert 1987; Hartnett et al. 1996), and incorporating these disturbance regimes into prairie restorations have been shown to benefit restored plant communities (Howe 1999; Tix and Charvat 2005)

Ecological succession is the directional change of species in a community, and associated changes in ecosystem processes based on an ecological time scale. Succession usually occurs following a disturbance, and depending on the magnitude of the disturbance, restoration can initiate, assist, and or accelerate the successional trajectory (Luken 1990). If the disturbance is minimal to moderate, then the community may be able to recover without intervention, but if the disturbance is severe (e.g. strip mine reclamation), then restoration efforts may be needed to aid natural successional processes.

Rationale for proposed research

The goal of many prairie restorations is to restore grass and forb species in proportions reflecting plant species diversity in native prairie. In tallgrass prairie restoration there is typically a target grass-forb species mixture in the initial seeding, but often over time the grasses become excessively dominant and forb species are underrepresented. A variety of research studies involving carbon additions, mowing, selective plant removals, and mycorrhizal manipulations have examined ways to increase forb diversity in newly restored grasslands (Smith et al. 1998; Bluementhal et al. 2003; Averett et al. 2004; Corbin and Antonio 2004; Siletti et al. 2004; Tix and Charvat 2005; Wilson 2002), but few studies have assessed the potential for enhancing forb cover and diversity in a well-established grass-dominated prairie restoration. The primary objectives of the investigations that follow were to: 1) examine the potential suppressive effects of dominant grasses on plant diversity and productivity by experimentally reducing the cover and biomass of two dominant grass species, *Andropogon gerardii* or *Panicum virgatum*; 2) assess the response of restored grassland communities (i.e. diversity and productivity) to long-term manipulation of soil resources (initiated in 1998) and to aboveground biomass removal via

mowing (initiated in 2005); and 3) determine the role of mycorrhizae in structuring plant communities during prairie restoration, since AM fungi have been shown to influence the composition of the aboveground plant community in native prairie (Hartnett and Wilson 2002). These objectives were addressed by: 1) examining aboveground (i.e. light availability, plant species cover and diversity) and belowground (i.e. nitrogen availability and soil moisture) responses to removal of the dominant grasses in a restored prairie; 2) experimentally manipulating the availability of soil resources (i.e. nutrient availability and soil depth) and aboveground biomass (i.e. mowed or unmowed) in a grass-dominated prairie restoration; and 3) experimentally manipulating root colonization by mycorrhizal fungi in order to assess the role of this plant-fungal symbiosis during introduction of forb species into a grass-dominated restored prairie. These studies addressed some fundamental questions concerning the controls of plant productivity and diversity in well-established long-term tallgrass prairie restorations. First, can the dominant grasses be manipulated to increase the presence and cover of the subordinate forb species? Second, what are the key factors regulating diversity and productivity? Third, are soil resource manipulations (i.e. nutrient availability and soil depth) still having an impact on ecosystem attributes (e.g. productivity, species composition, and soil nutrients) 7-9 years after the initial restoration? Fourth, can the suppression of the mycorrhizal symbiosis reduce the cover or productivity or both of dominant grasses to a level which allows subordinate species to establish? Lastly, what environmental factors are most important in influencing the recovery and establishment of forb species in a long-term grass-dominated prairie restoration? Together, these investigations aim to provide new data that can be used to promote recovery of plant species in restored prairies, which in turn should improve ecosystem structure (e.g. diversity) in restored grasslands.

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CHAPTER 2 - DOMINANT GRASS REMOVAL INCREASES PLANT DIVERSITY IN RESTORED TALLGRASS PRAIRIE

Abstract

A common outcome of tallgrass prairie restorations is the successful establishment of the dominant C_4 grasses, while richness and abundance of forb species are often slow to recover, leading to low plant diversity in restored communities. In order to enhance the diversity of restored grasslands, it may be necessary to incorporate management strategies that reduce the dominance of C_4 grasses. In order to determine if competitive interactions with the dominant grasses limit plant diversity, we established an experiment where two dominant grasses *Andropogon gerardii* or *Panicum virgatum* were physically removed from plots through clipping and foliar herbicide application. Abundance of each species was reduced by either 50% or 100% relative to unmanipulated control plots. In the control plots, *A. gerardii* was the most abundant grass with up to 80% cover, while *P. virgatum* had up to 40% cover.

Number of species present was inversely correlated with grass productivity and percent grass cover in 2006, and positively correlated with light availability. The positive relationship between species richness and light availability as well as the negative relationships between species richness and grass productivity and cover suggested that differences in species richness among the removal treatments resulted from treatment induced differences in aboveground resources rather than the belowground resources. Removing the more abundant *A. gerardii* significantly increased light availability in year one, and after three growing seasons increased forb productivity, forb cover, species richness, species evenness, and species diversity compared to unmanipulated control plots, while removing the less abundant *P. virgatum* did not significantly affect these parameters. These positive responses to the removal of *A. gerardii* suggested a competitive release of the subordinate forb species. In conclusion, after three growing seasons it appears that competitive interactions between dominant grasses and forb species do limit forb cover, forb abundance, and species richness in this restored tallgrass prairie.

Therefore, management practices that target reductions in cover or biomass of dominant grasses may be useful for enhancing diversity in restored grasslands.

Key Words: *Andropogon gerardii*, competition, diversity, dominant grasses, removal, restoration, tallgrass prairie

Introduction

For more than 50 years, prairie restorations have been implemented to recover representative plant and animal communities characteristic of the once extensive North American tallgrass prairie ecosystem (Sperry 1990). Since the 1830s, an estimated 82-99% of the native North American tallgrass prairie has been lost (Samson and Knopf 1994) due to conversion to agriculture, altered disturbance regimes (e.g. fire suppression), fragmentation and exotic species invasion (Hoekstra et al. 2005). This decline represents the greatest loss of any one North American terrestrial ecosystem type since European settlement (Samson and Knopf 1994), and accentuates the need to design and implement better methods to restore the structure and function of this degraded ecosystem (Webb 1996).

The goal of most prairie restorations is to restore both dominant and subordinate plant species in proportions that reflect plant species diversity in native prairies, but many restoration attempts fall short of matching the species diversity of their native counterparts (Thompson 1992; Howe 1994, 1995, 1999; Kindscher and Tieszen 1998). In tallgrass prairie restoration, the initial seeding typically includes a target grass-forb species mixture; however, the warm-season (C_4) grasses often become dominant shortly after establishment (Warkins and Howell 1983; Sperry 1994; Kindscher and Tieszen 1998) while it is more difficult to establish and maintain subordinate grass and forb species (Schramm 1976; Sperry 1983; Warkins and Howell 1983) leading to overall low species richness and diversity. If dominance by these grasses is permitted to increase further, many subordinate species will eventually disappear (Howe 1999).

In native tallgrass prairie, the dominant C_4 grasses have a disproportionate influence on ecosystem processes such as primary productivity (Smith and Knapp 2003) and exert strong competitive effects on subordinate species, which enhances their influence in structuring tallgrass prairie plant communities (Collins 1987; Gibson and Hulbert 1987; Hartnett and Fay 1998; Hartnett and Wilson 1999, 2002). Several studies in native prairie have shown that

manipulating factors that alter the abundance or productivity of the dominant grasses may result in competitive release for subordinate grass and forb species (Collins 1987; Hartnett et al. 1996; Silletti et al. 2004). Collins (1987) altered disturbance regimes (fire and cattle grazing) in native tallgrass prairie and concluded that burning and grazing differentially affected the dominant grasses, resulting in unique, contrasting changes in subordinate species abundance and community structure. Grazing decreased cover of *Andropogon gerardii* and increased subordinate species cover, while fire increased cover of *A. gerardii* which, in turn, resulted in a decrease in cover of subordinate species (Collins 1987). Hartnett and others (1996) also concluded that grazing by bison decreased *A. gerardii* which led to an increase in subordinate species. The role of interspecific competition in regulating plant community structure in native tallgrass prairie also has been assessed with experimental removal studies. Silletti and others (2004) removed two dominant grasses (*A. gerardii* or *Sorghastrum nutans*) independently from experimental plots in native grass prairie and concluded that the codominant species, *S. nutans*, increased performance (net photosynthesis, stomatal conductance, and tiller mass) when *A. gerardii* was removed. Understanding how native plant community structure changes in response to manipulation of the dominant species (e.g. *A. gerardii*) is likely to be beneficial in predicting how restored communities will respond to dominant species manipulation and management.

Fewer studies have addressed the potential role of dominant species in limiting diversity in restored grasslands. Previous research studies have examined soil and plant responses to altered resource availability through nutrient manipulations, soil depth alterations, mowing, grazing, selective plant species removal, and mycorrhizal manipulations in order to design more effective methods to increase forb diversity on newly restored areas (Smith et al. 1998; Howe 1999; Wilson 2002; Baer et al. 2003, 2004; Blumenthal et al. 2003; Averett et al. 2004; Corbon and Antonio 2004; Tix and Charvat 2005). However, less research has been done to assess the potential for enhancing forb cover and diversity in well-established prairie restorations that have already become grass dominated.

The purpose of this study was to examine the effects of dominant grass species removal on community structure (species richness, evenness, diversity) and function (soil nitrogen availability and ANPP) in a prairie restoration established in 1998, seven years prior to the start of this study. The dominant vegetation at the start of this study consisted of two warm-season

(C_4) perennial grasses, *Andropogon gerardii* Vitman (big bluestem) and *Panicum virgatum* L. (switchgrass), with *A. gerardii* more abundant (up to 80% cover) relative to *P. virgatum* (up to 40% cover). Previous removal studies have examined the effects of competition with dominant species on the whole plant community and provided indirect evidence of subordinate species and overall diversity being suppressed by competition with the dominant species (Wardle et al. 1999). These studies were done primarily in old fields and other native terrestrial systems (Pinder 1975; Allen and Forman 1976; Abul-Fatih and Bazzaz 1979; Hils and Vankat 1982; Armesto and Pickett 1985, 1986; Gurevitch and Unnasch 1989; Smith and Knapp 2003), but the potential for increasing species diversity by removing or reducing dominant plant species during restoration has not been well investigated. In order to address the potential suppressive effects of the dominant grasses on the plant community, we removed either 50% or 100% of *A. gerardii* or *P. virgatum* to test the following hypotheses: (1) reducing the dominant grasses will increase resource availability (i.e. light, N availability, and soil moisture), (2) competition with the dominant grasses limits subordinate species (i.e. forbs) in restored tallgrass prairie and removal of the dominant grasses will increase abundance and cover of forbs, and species richness and diversity, and (3) removing *A. gerardii* will have a greater effect on resource availability (i.e. increased light availability, N availability, and soil moisture) and species diversity due to this species being more abundant than *P. virgatum.*

Materials and Methods

Study site

Research plots were located in a prairie restoration experiment that was established in 1998 (see Appendix 1 for plant species and seeding rates) in a former lowland agricultural field at Konza Prairie Biological Station (KPBS), a 3487 ha tallgrass prairie preserve located in the Flint Hills region of Northeastern Kansas (39°5´N, 96°35´W). Mean annual precipitation at the site is 834 mm yr^{-1} (1891-2002) with high variability between years (coefficient of variation = 24%); approximately 635 mm falls during the growing season (April through September) of each year (Sophocleous 1998). In the three years of study (2005, 2006, 2007) total precipitation was 959, 631, and 693 mm, of which 707, 570, and 607 mm fell during the growing season (April through September) of each year, respectively. Even though total rainfall in 2006 was near average, 301 mm occurred late in the growing season (August through September) and water

stress was evident through much of the growing season. Prior to restoration, the site had been cultivated for more than 50 years. The soil was a Reading silt loam (mesic Typic Argiudoll) formed by alluvial and colluvial deposits. Following initiation of the restoration experiment, the area became dominated by a few C_4 grass species, predominantly *Andropogon gerardii* Vitman and *Panicum virgatum* L., with *Sorghastrum nutans* (L.) Nash being relatively common (nomenclature follows USDA, NRCS Plants Database [2007]). The remaining plant community consisted primarily of a few forb species, including *Salvia azurea* Michx. ex Lam., *Baptisia australis* (L.) R.Br. ex Ait. f. var. *minor* (Lehm.) Fern., *Lespedeza capitata* Michx., *Brickellia eupatorioides* (L.) Shinners, and *Vernonia fasciculata* Michx. The entire restored field, including the experimental plots, was burned frequently with prescribed spring fires.

Establishment of experimental plots

In May 2005, thirty 1 × 1 m plots (with 0.83-m buffer strips between all plots) were delineated in a random complete block design. The entire site was burned several weeks prior to plot establishment. Six blocks were established 8-m apart, and four removal treatments plus one untreated control were assigned randomly to each plot within a block. Thus, each treatment was replicated six times. In each block, one plot each was assigned to 100% *A. gerardii* removal (AG100), 50% *A. gerardii* removal (AG50), 100% *P. virgatum* removal (PV100), 50% *P. virgatum* removal (PV50), and an untreated control. For the removal treatment, individual grass tillers were clipped and the grass-specific herbicide Ornamec™ (Fluazifop-P-butyl: Butyl (R)-2-[4[[5-trifluoromethyl)-2-pyridinyl]oxy]phenoxy] propanoate; PBI/Gordon Corporation, Kansas City, Missouri) was applied to each clipped tiller using a small sponge, carefully avoiding contact with the soil or other non-target species. The 50% removal treatments were achieved by clipping and herbiciding every other target grass tiller encountered in a systematic sweep of the plot in order to maintain a relatively natural distribution of tillers in each plot. Due to differences in the initial abundance of the two target dominant grasses, the total amount of biomass removed from each plot during the herbicide treatment was different, e.g. the 100% *A. gerardii* removal plots had more biomass removed then the 100% *P. virgatum* removal plots. Due to the nature of the project and the coverage of the grasses, it was not feasible to keep the total amount of biomass removed constant between *A. gerardii* and *P. virgatum* removal plots. In addition, the objectives were to assess the relative impacts of the removal of the two most dominant grasses in

proportions that reflected their actual abundance in the field. Removals were initially performed in early May 2005. Plots were checked periodically through July 2005 and any re-growth of treated individuals of a target species was removed in the same manner. The removal treatments did not need to be repeated in the following years.

Belowground sampling

Soils were sampled for extractable inorganic N (NO_3^- and NH_4^+) in midseason (late June to early July) and in late season (September) for 2005 and 2007. In 2006 soils were sampled monthly during the growing season from May to September to assess temporal patterns of N availability and potential seasonal differences among treatments. Two soil cores (10 cm deep x 2 cm diameter) were collected and composited from each 1 × 1 m plot, crumbled by hand and sieved through 4-mm mesh to remove roots and rocks, and stored at 4˚C until being extracted. Inorganic N was extracted from samples (11-12 g field moist soil) using 2 mol·L^{-1} KCl, and extracts were filtered through 0.4-µm polycarbonate filters (Osmonics Inc.). Extracts were analyzed colorimetrically for NO_3-N and NH_4-N on an Alpkem Flow Solution® autoanalyzer (OI analytical, College Station, Texas, USA). Nitrate (NO_3-N) was determined by diazotization with sulfanilamide after reduction through a cadmium coil, and ammonium (NH_4-N) was measured using the phenol blue method (Keeney and Nelson 1982). The remaining soil was weighed field moist, dried for 2 days at 60°C, and reweighed to determine gravimetric soil water content.

In 2007, ion exchange resin bags were buried in each plot to provide another index of relative inorganic N availability (Binkley and Hart 1989). Bags were constructed of nylon mesh material and filled with a 1:1 mixture (10 g total) of cation exchange resin (Dowex HCR-W2) and anion exchange resin (Dowex 1 X 8-50) pre-loaded with H^+ and Cl^-, respectively. One resin bag was buried in the surface 10 cm in each plot in late May and harvested in September. In the laboratory, resin bags were rinsed under running deionized water to remove excess soil, and extracted with 100 mL of 2 mol·L^{-1} KCl by shaking for 2 hours at 200 rpm. Extracts were then filtered and analyzed using the Alpkem Flow Solution® autoanalyzer (OI analytical, College Station, Texas, USA) as described above. Since the resin bag extracts were acidic, all samples were neutralized prior to analysis.

Aboveground sampling

Light availability

In July 2005, mid-season percent light transmission through the plant canopy was quantified in all plots. In 2006 and 2007, light transmission was recorded monthly throughout the growing season. All measurements were made at midday (1100-1300, Central Daylight Time) under full sun conditions. Three measurements of photosynthetic photon flux density (PPFD) ($\mu mol \cdot m^{-2} \cdot s^{-1}$) were taken in each plot. One measurement was made above the plant canopy and two orthogonal measurements were made at the soil surface with a 0.5 m Sunfleck ceptometer (Decagon, Pullman, Washington). The two soil surface measurements were averaged for each plot, and light transmission was expressed as percent PPFD reaching the soil surface.

Community indices

In all years, percent cover of each plant species was visually assessed in spring (late May-early June) and summer (August) for all plants rooted within a 0.25-m^2 quadrat in each plot. For each species, the maximum cover value from the combined spring and summer sample dates was used to calculate plant species richness, diversity, and evenness. Species richness (S) was calculated as the number of plant species per 0.25-m^2 quadrat. Diversity was calculated for each plot using Shannon's diversity index, $H' = -\Sigma p_i \ln p_i$, where p_i represented the proportion of total cover contributed by species *i*. Shannon's diversity index was selected because it includes proportional representation of species in a community and provides relatively even weighting to both richness and evenness (Barbour et al. 1999). Evenness was calculated using Pielou's index, $J = H'/H_{max}$, where H_{max} represented the natural log of S. Plant species richness, evenness, and diversity were calculated including and excluding the manipulated removal species (i.e. *A. gerardii* or *P. virgatum*) from the treated removal plots as well as from the untreated control plots.

Biomass

Near the end of each growing season (late August, early September), aboveground biomass was harvested from each plot in an area outside the species composition sampling quadrat ($n = 30$). Vegetation from one 0.25-m^2 area in each plot was clipped at ground level and sorted into the following categories: live grass, live forb, and surface plant litter. In 2007 only,

biomass was sorted by grass species. The surface plant litter was minimal due to annual spring burning during the study period, and was not separated into grasses or forbs; therefore, it was excluded in calculating grass and forb productivity, but was included for calculating total aboveground net primary productivity (ANPP) by plot. Biomass was oven-dried at 60°C for at least 48 hours, and weighed separately by category, then summed to estimate ANPP, a measure of ecosystem function (Briggs and Knapp 1995). Annual productivity estimates represented only biomass produced in the year of measurement, since all treatment plots were burned annually during the period of study.

Statistical analyses

Resource availability (soil moisture, N and light), plant cover, productivity, and community indices (species richness, evenness and diversity) were analyzed according to a random complete block design. Plant community responses to removal treatments were analyzed by year and across all years with repeated measure analysis. In 2006, resource availability (N and light) was assessed monthly, and repeated measures analysis was used to determine treatment differences in resource availability across the growing season. All data were analyzed using mixed-model analysis of variance (SAS Version 9.1; SAS Institute Inc. 2002-2003), with block as a random factor and removal treatment as a fixed factor. Denominator degrees of freedom were estimated using the Satterthwaite's method for all tests of fixed effects (treatment, time and treatment × time). All means comparisons were performed using the difference in least squares means procedure, $\alpha = 0.05$ (SAS 2002-2003). Relationships between response variables were examined using correlation analyses (r = Pearson correlation coefficient) in SAS, $\alpha = 0.05$.

Results

Productivity and cover

Although total ANPP averaged across treatments varied by year, there was no effect of the removal treatments on total ANPP across or within years (Table 2-1). Grass ANPP was significantly reduced by the *A. gerardii* removal treatments across all years and within years, with the greatest reduction in the 100% *A. gerardii* removal treatment (Table 2-1). The *P. virgatum* removal treatments did not affect grass ANPP across all years, though grass ANPP was

significantly reduced in the 100% *P. virgatum* removal treatment in 2005. The removal of *A. gerardii* increased forb ANPP in all years, with 100% *A. gerardii* removal increasing forb ANPP the most compared to other treatments (Table 2-1). Removal of *P. virgatum* did not significantly alter forb ANPP. When comparing ANPP in individual years across treatments, forb, grass and total ANPP were significantly lower in 2006 compared to other years (Table 2-1). Figure 2-1 illustrates the contribution of *A. gerardii*, *P. virgatum* and other grass species to end-of-season total grass ANPP in 2007. *Andropogon gerardii* comprised the majority of grass ANPP in the control treatment and was significantly reduced in the 50% and 100% *A. gerardii* removal treatments compared to the other treatments (top panel Figure 2-1). *Andropogon gerardii* biomass increased by more than 50% in the 100% *P. virgatum* removal treatment, relative to the control, though the difference was not statistically significant. *Panicum virgatum* biomass was significantly reduced in the 50% and 100% *P. virgatum* removal treatments compared to the control (middle panel Figure 2-1). For the remaining grasses (primarily *Sorghastrum nutans*) there were no differences among treatments, although other grass biomass was greatest in the 100% *A. gerardii* removal treatment and lowest in the *P. virgatum* removal treatments (bottom panel Figure 2-1). These results suggest that *P. virgatum* removals may be suppressing these other grasses while the 100% *A. gerardii* removal may be enhancing these other grasses (Figure 2-1).

In 2005, the grass species cover in the control plots consisted of *A. gerardii* (70.0 % ± 10.08 cover), *P. virgatum* (35.0 % ± 5.47 cover), and *S. nutans* (5.5% ± 0.96 cover). Across and within all years, grass cover was significantly less in *A. gerardii* removal plots compared to the control, with 100% *A. gerardii* removal having the least grass cover (Table 2-2). The removal treatments generally increased forb cover compared to the control. Removal of *A. gerardii* enhanced forb cover in all years, with 100% removal of *A. gerardii* resulting in significantly higher coverage of forbs in all years (Table 2-2). Effects of *P. virgatum* removal varied with percent removal and year, though 50% *P. virgatum* removal increased forb cover across years. Total percent plant cover was reduced by all removal treatments in 2005, the year the removal treatments were implemented (Table 2-2). However, there were no differences in total plant cover among treatments by the next year (2006), which may have been due to compensatory increases in forb species cover, specifically *Baptisia australis* (L.) R. Br., *Salvia azurea* Michx. Ex Lam., and *Lespedeza capitata* Michx, in the removal treatments. Figure 2-2 illustrates grass

cover detailed by species for all three years. There were increases in cover of *S. nutans* by 2007 in both *A. gerardii* removal treatments, though differences among treatments were not significant.

Community indices

For all community indices there was no interaction between year and removal treatment. For the whole community indices (including the grass species targeted for removal) species richness, evenness and diversity did not differ among years (Table 2-3A). However, when compared across all years, 50% and/or 100% removal of *A. gerardii* resulted in significant increases in species richness, species evenness (only in 50% removal of *A. gerardii*), and species diversity (Table 2-3A). In 2005, species richness was not different among treatments, but by 2007 both *A. gerardii* removal treatments had significantly greater richness compared to the control and *P. virgatum* removal treatments. Within a given year, there was a variable response in species evenness to removal treatments, while across all years only the 50% *A. gerardii* removal treatment had greater evenness than other treatments (Table 2-3A). Species diversity of the whole community was increased each year with 50% and/or 100% removal of *A. gerardii.*

The community responses were also analyzed with target grass species excluded from the calculations of community indices in order to de-emphasize the effects of the manipulations *per se* and to determine how the remaining community responded to dominant grass removals (Table 2-3B). Across all treatments species richness in 2006 was less than 2005 and 2007 ($p = 0.0155$, Table 2-3B). A similar pattern occurred for species diversity, but differences among years were not significant. Species evenness was not significantly different among years, but there was a trend for increasing evenness over time (Table 2-3B). Both the 50% and 100% removal of *A. gerardii* increased species richness, species evenness and species diversity of the remaining plant community; however, this did occur for removal of *P. virgatum* (Table 2-3B). Changes in species richness appeared to accrue over time, while significant differences in species evenness and diversity were evident in the first year following removals. Overall, the plant community appeared to respond more positively (i.e. increased values for species richness, evenness, and diversity) to the removal of *A. gerardii* than to *P. virgatum* removal. Even though species richness and diversity increased in the *A. gerardii* removal treatments, it is worth noting that

plant communities in this restoration remained much less diverse than comparable areas of native prairie on similar slope and soil conditions (Table 2-3).

Whole community species richness was positively, though weakly, correlated with light availability, and inversely correlated to grass productivity and percent grass cover in 2006 (Figure 2-3). The positive relationship between richness and light availability as well as the negative relationships between richness and grass productivity and cover suggested that differences in richness among the removal treatments may be linked to treatment induced differences in aboveground resources rather than the belowground resources. Removing a subset of the most dominant grasses increased richness. This suggested that subordinate forb species benefited from a competitive release when dominant grasses were reduced. However, there were no significant correlations between diversity and light availability, grass productivity, or grass cover. The correlations between evenness and light availability, grass productivity, or grass cover also did not show strong relationships, suggesting that increases in diversity observed were being driven by species richness rather than evenness.

Resource availability

Aboveground resources

The removal treatments initiated in 2005 effectively increased light availability for the duration of this study (Figure 2-4). The repeated measures analysis resulted in significant main effects of removal treatment (Figure 2-4a) and sampling date (Figure 2-4b), but there was no significant sampling date × treatment interaction. When examining the effects of removal treatment across all dates, the 100% removal of *P. virgatum,* and 50% and 100% removal of *A. gerardii* resulted in more light reaching the soil surface compared to the control plots and the 50% *P. virgatum* removal treatment ($F = 10.02$, $p < 0.0001$). Specifically, the 100% removal of *A. gerardii* had the greatest light availability averaged across sample dates (Figure 2-4a). In 2006 and 2007, light availability was greatest early in the growing season, and decreased as the growing season progressed ($F = 20.84$, $p < 0.0001$; Figure 2-4b).

Belowground resources

Removal treatments had no significant effects on early or late growing season inorganic N or soil moisture within any year (Table 2-4), but there were differences among years for

inorganic N averaged over all treatments (Table 2-4). Since early and late growing season samples produced similar trends, only late growing season results were presented in Table 2-4. In 2006, monthly soil cores were collected and figure 2-5 illustrates the temporal trends in soil resource availability across all sampling dates for total extractable inorganic N (A) and percent soil water content (B). These parameters showed significant main effects for sampling date only with nitrogen availability ($F = 20.25$, $p < 0.0001$) and soil water content ($F = 610.65$, $p < 0.0001$). These parameters were significantly less during mid-growing season (Figure 2-5) while light availability was greatest early in the growing season (Figure 2-4b). This corresponds directly to plant growth and reproduction. As plants grow they decrease light availability reaching the soil surface and often reduce concentrations of inorganic soil nitrogen as N is assimilated to support plant growth. As plants begin to senescence at the end of the growing season, and soil water content typically increases, concentrations of inorganic soil nitrogen may begin to increase.

Discussion

Treatment effects on resource availability

The data support the hypothesis that removing or reducing the dominant grasses will increase resource availability in restored grasslands, but only with respect to aboveground resources. Removing the dominant grasses, specifically *A. gerardii*, led to increased light penetration through the canopy. However, the removal treatments did not alter belowground resource availability (N), which differs from other studies that have demonstrated increased soil nutrient availability with neighbor removals in an old field (Symstad and Tilman 2001) and in an Alaskan tussock tundra (Bret-Harte et al. 2004). However, the lack of change in available soil N observed in this study was comparable to a dominant grass removal study conducted on native tallgrass prairie (Silletti et al. 2004) where removal of all *A. gerardii* or *S. nutans* from plots did not significantly change soil NO_3-N compared to control plots. The similarity in belowground resources among treatments in the present study may also have been impacted by the prior long-term agricultural management at this site. In general, agricultural fields have a history of fertilizer use which increases soil nutrient availability compared to native prairie soil. Total inorganic N at the end of the growing season significantly decreased each year of this study and by 2007 the values were comparable to end-of-season total inorganic N for native prairie (2-3 μg

N g soil^{-1}; McKinley 2007). Since soil C and N pools in this restoration are becoming more similar to native prairie (Baer and Blair 2008), responses of belowground resources to removal treatments may occur in the future. However, since belowground resources were not significantly affected by removal treatments in this study, it is reasonable to assume that the changes in community structure observed in response to dominant species removal were driven by changes in aboveground resources (i.e. light availability) and altered competitive interactions between the dominant grasses and forb species.

Treatment effects on plant community structure

The data support the hypothesis that competition with the dominant grass species *A. gerardii* limits other species in this restoration. Removal of *P. virgatum* did not produce the same results. Although *P. virgatum* was the most dominant grass early in the restoration, and was associated with reduced species richness and diversity (Baer et al. 2004), its cover had dropped to < 40% by the start of this study, while cover of *A. gerardii* had increased. Removal of the more dominant *A. gerardii* resulted in increased species richness, evenness and diversity after three years. In addition, forb species increased in biomass, cover, or number in response to the removal of *A. gerardii*. This suggests that removing the dominant grasses lessened competition and provided a competitive release for the subordinate species. We are aware of no comparable studies that have examined the effects of dominant species removal on community structure in established restorations, but our results were comparable to other dominant species removal studies conducted in old fields and other native plant communities. Our results are consistent with those of Gurevitch and Unnasch (1989) in a two-year study removing the dominant species *Dactylis glomerata* L. from an old field. This study showed that removing *D. glomerata* increased species diversity, evenness, and species richness with subordinate species increasing in abundance or frequency (Gurevitch and Unnasch 1989). Several other studies examining species removal effects on community structure were conducted for only one year, therefore comparisons of community responses over multiple years cannot be made. In these single-season studies, the effects of species removals on community structure were variable. Three of the five studies found that dominant species removal did not significantly increase or decrease species diversity or species richness (Pinder 1975; Allen and Forman 1976; Hils and Vankat 1982). In the other two studies (Abul-Fatih and Bazzaz 1979; Armesto and Pickett 1985,

1986), species diversity was increased with removal of the dominant species. Smith and Knapp (2003) removed dominant grass species over two growing seasons in a native grassland resulting in decreased ANPP, decreased grass cover and increased light availability, but the production of the subordinate species was unaffected and not enhanced through competitive release. They concluded that even with increased light and space availability, the subordinate species were unable to take advantage of the increased resources due to possible stressful microclimate conditions (e.g. increased evapotranspiration, decreased soil moisture).

Even though we did not test the core-satellite hypothesis (Hanski 1982) directly in this study, it may provide another explanation for the patterns we observed. The core-satellite hypothesis (Hanski 1982) explains the dichotomous distribution between the abundant widely distributed ("core") species and rare patchily distributed forb ("satellite") species. Gotelli and Simberloff (1987) confirmed that the patterns in distribution and abundance of tallgrass prairie plants support the core-satellite hypothesis, where the "core" species include the C_4 grasses and the "satellite" species include the subordinate grasses and forbs. Evidence indicates the matrix of dominant grasses in the tallgrass prairie are regulated by competition, while the satellite species non-equilibrium patch dynamics are influenced by disturbances operating at different scales, and patterns of species richness in tallgrass prairie are driven by these non-equilibrium dynamics of the satellite species (Hartnett and Fay 1998). The core-satellite hypothesis provides another explanation, besides competition alone, for an alternative mechanism influencing species diversity in this study.

Conclusion

Native tallgrass prairie plant communities are usually dominated by a few grass species intermingled with a large number of varying subordinate species that occur in low abundance (Gotelli and Simberloff 1987), and which comprise the bulk of species diversity of these communities (Collins and Glenn 1990). During restoration, excessive dominance of the grasses commonly becomes a problem resulting in overall low species richness and diversity. Manipulating factors that decrease grass dominance may provide competitive release for the subordinate species (Collins 1987; Hartnett et al. 1996; Howe 1999), and an opportunity for them to increase in abundance or cover or both. It appears that in order to achieve and maintain the desired level of diversity in tallgrass prairie restorations, the dominance of the native grasses

may need to be inhibited (Baer et al. 2005). Methods to control dominant grass species during restoration include variable fire regimes, mowing, and/or grazing (Howe 1994, 1995, 1999). While targeted species removals, as done in this study, would not be practical in a large-scale restoration, our results suggest that other approaches that reduce the abundance and cover of a dominant species, and increase canopy openness and light availability, may benefit subordinate forb species and enhance plant species diversity in restored grasslands.

Using an experimental approach in the context of restoration, such as in this study, provides an opportunity to test ecological theory in a novel setting with potential application to development of future restoration approaches (Bradshaw 1987; Howe 1999). Overall, the changes in patterns of diversity that we observed after three growing seasons are consistent with predictions of dominance-diversity relationships, with a clear linkage between decreased abundance and cover of dominant species and increased species richness and diversity. In addition, our results are pertinent to competition theory, with dominant grass species removal leading to the apparent competitive release for subordinate forb species. Thus, understanding how the subordinate species compete with the dominant species can be useful in guiding tallgrass prairie restoration.

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Figures and Tables

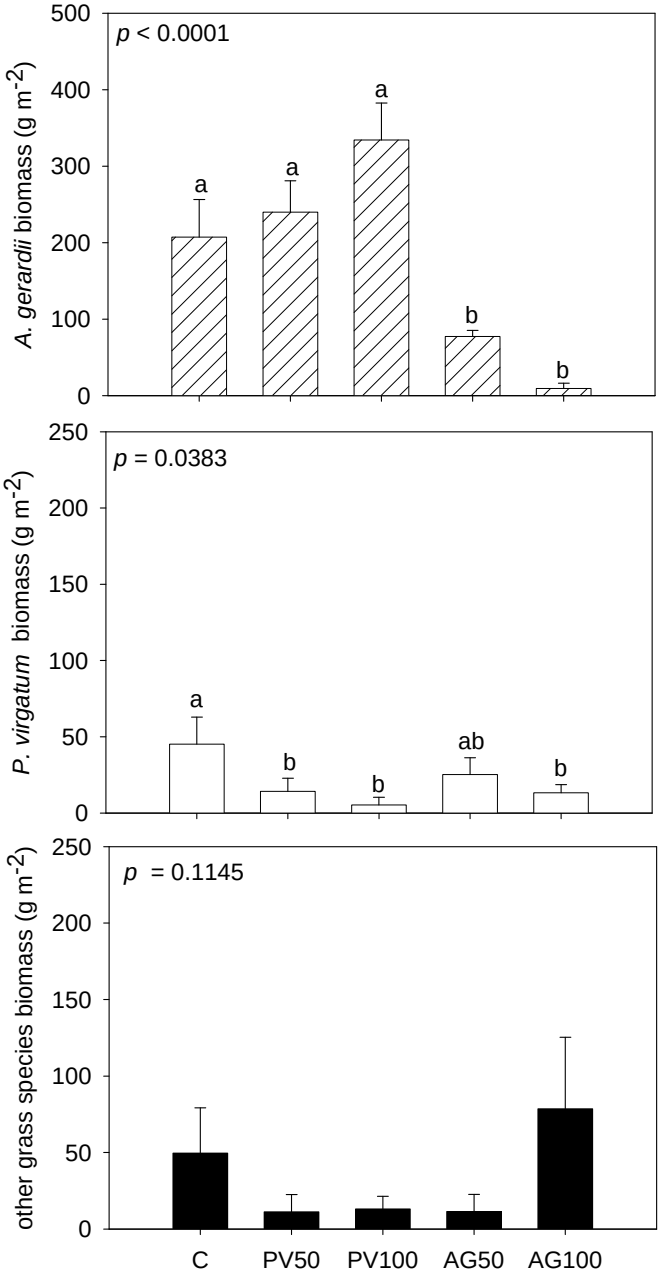


Figure 2-1. Mean (± SE) grass biomass (g m^{-2}) by species (*Andropogon gerardii*, *Panicum virgatum*, and other grass species) in 2007. Treatments included control (C), 50% *P. virgatum* removal (PV50), 100% *P. virgatum* removal, 50% *A. gerardii* removal (AG50), and 100% *A. gerardii* removal (AG100) (n=6 for each treatment). Means accompanied by the same letter were not significantly different ($\alpha = 0.05$). Note different scales on y-axes.

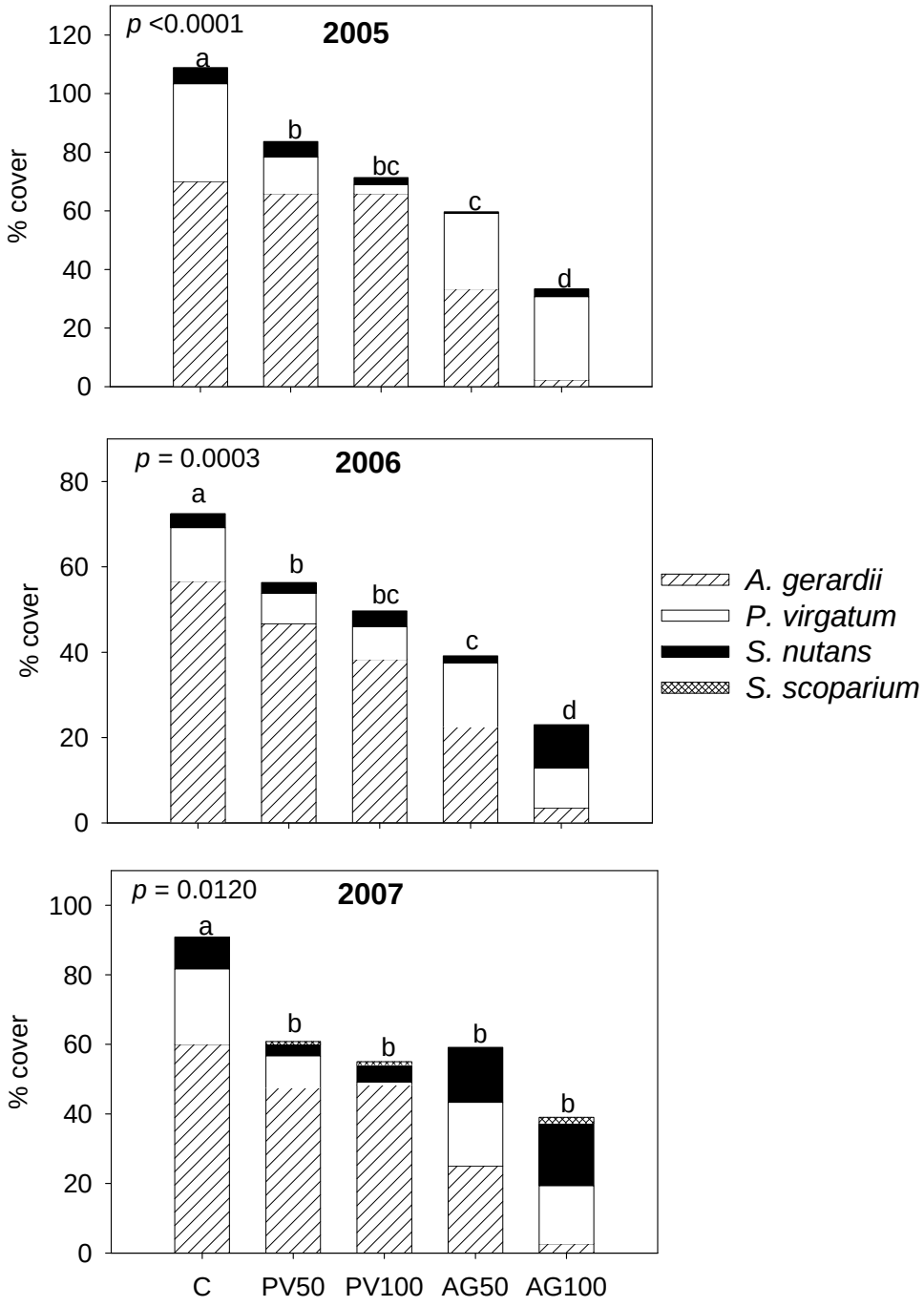
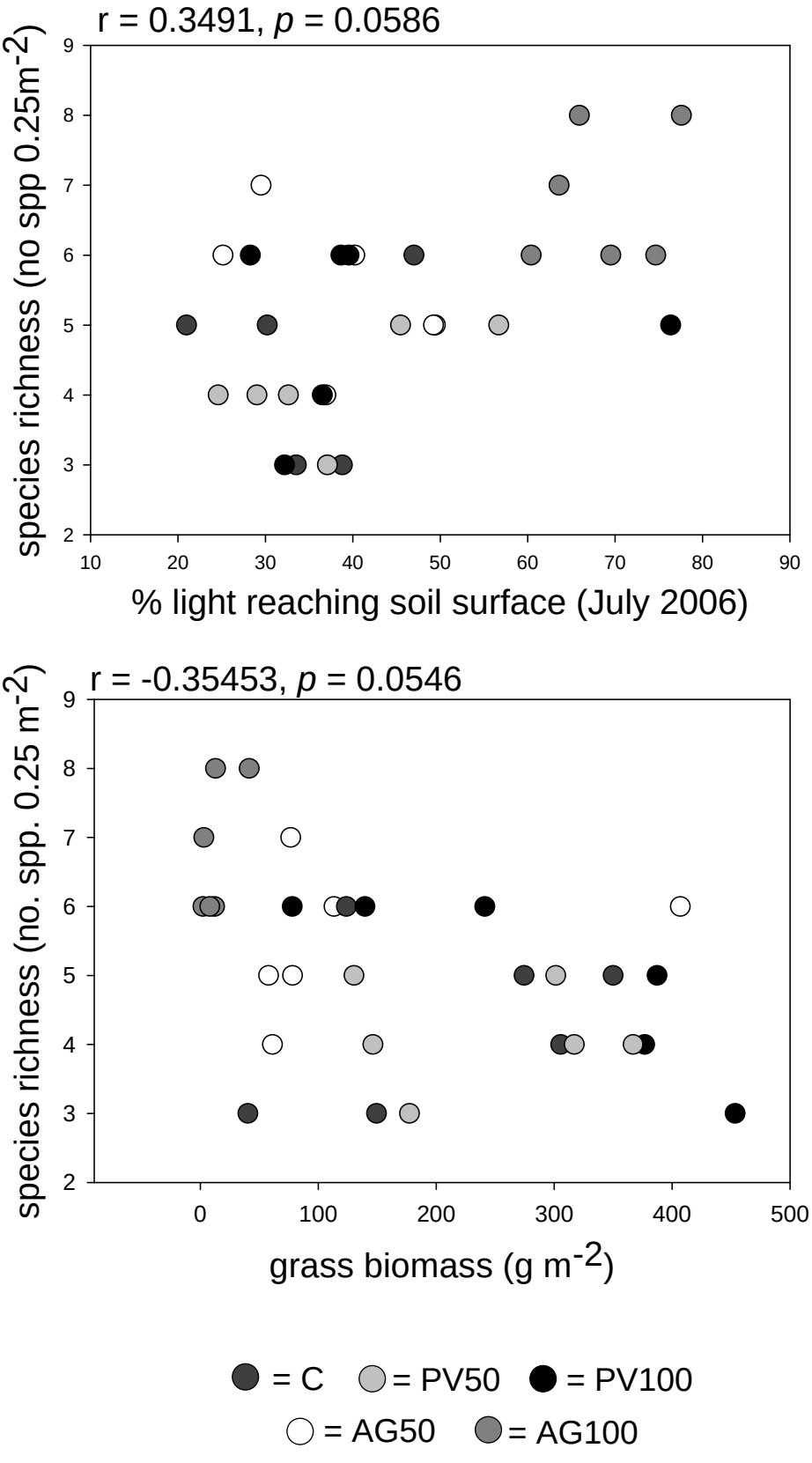


Figure 2-2. Means of percent cover by grass species (*Andropogon gerardii, Panicum virgatum, Sorghastrum nutans,* and *Schizachyrium scoparium*) for three years. Treatments included control (C), 50% *P. virgatum* removal (PV50), 100% *P. virgatum* removal (PV100), 50% *A. gerardii* removal (AG50), and 100% *A. gerardii* removal (AG100) (n=6 for each treatment). *P*-values correspond to total grass cover. Means accompanied by the same letter were not significantly different for total grass cover ($\alpha = 0.05$).


r = 0.3491, p = 0.0586
species richness (no spp 0.25m-2)
% light reaching soil surface (July 2006)
r = -0.35453, p = 0.0546
species richness (no. spp. 0.25 m-2)
grass biomass (g m-2)
= C
= PV50
= PV100
= AG50
= AG100

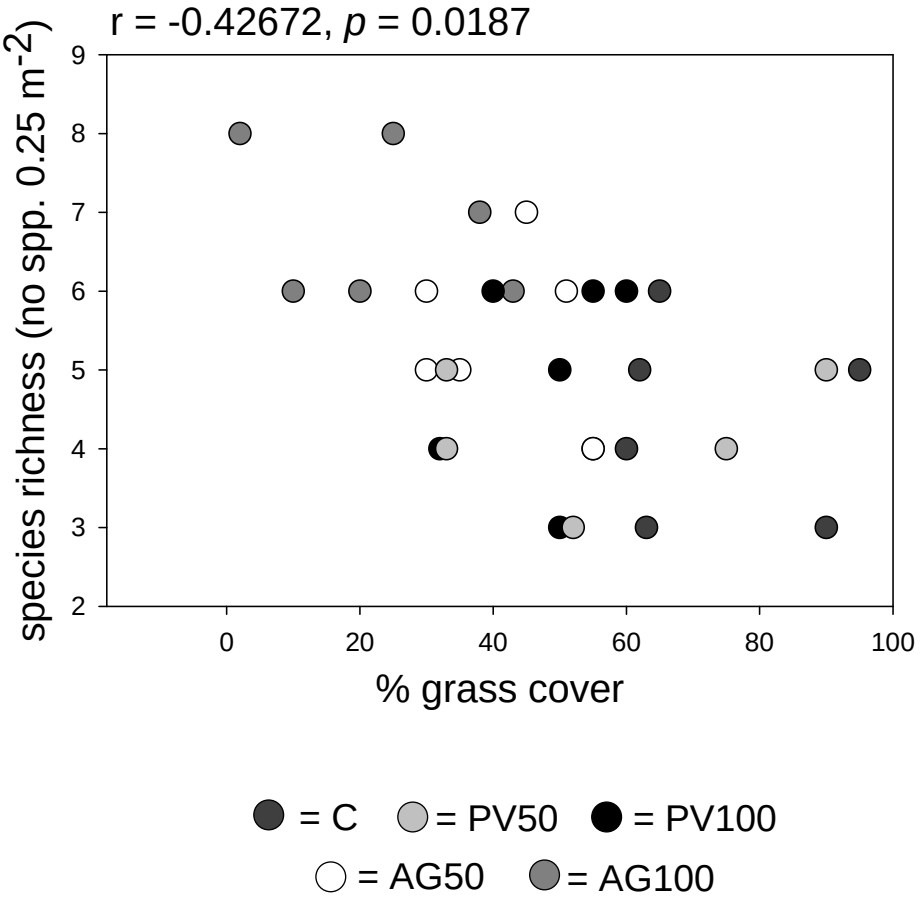
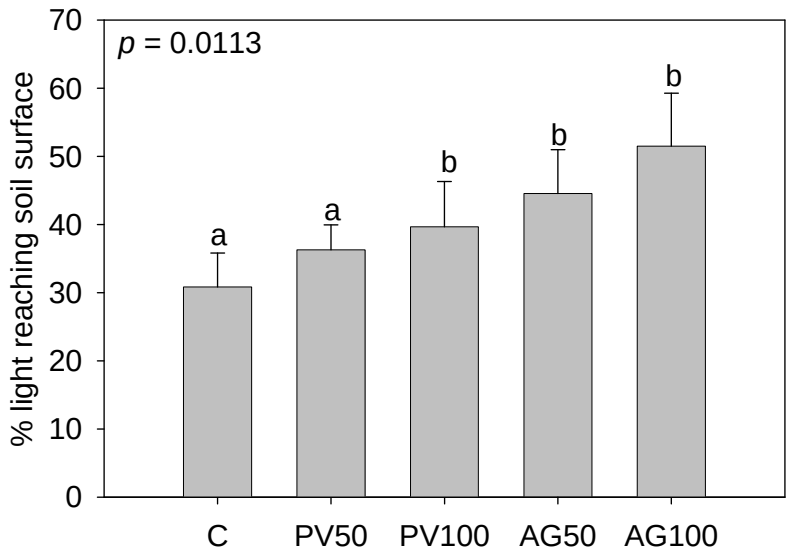


Figure 2-3. Correlations between total species richness and July light transmission (% light reaching the soil surface), grass biomass and % grass cover for 2006. Correlations were performed on an individual plot basis (n = 30) with removal treatments indicated with different symbols (C= control, dark grey circle; PV50 = 50% *P. virgatum* removed, light grey circle; PV100 = 100% *P. virgatum* removed, black circle; AG50 = 50% *A. gerardii* removed, white circle; and AG100 = 100% *A. gerardii* removed, medium grey circle). Significant relationships were determined from the Pearson correlation coefficient (r) derived using SAS (SAS 2002-2003).

A.



B.

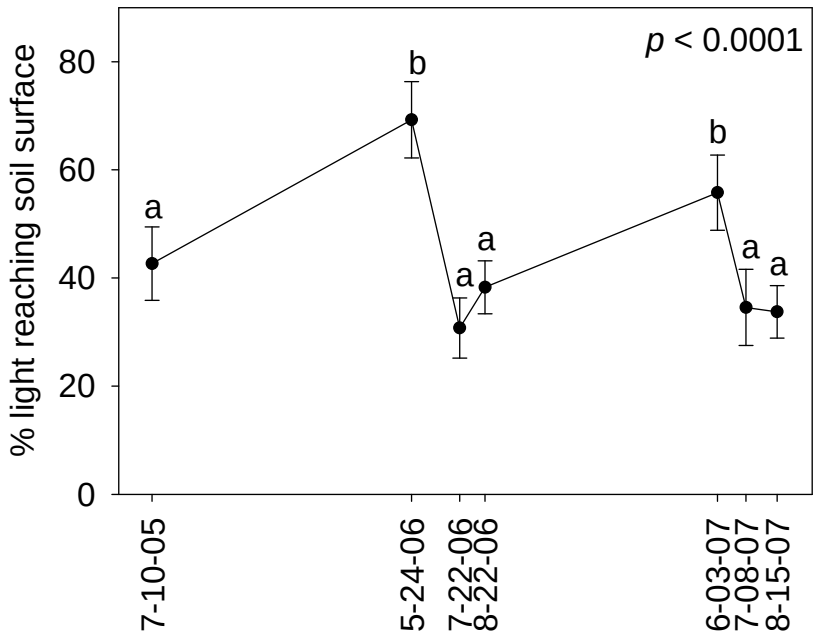
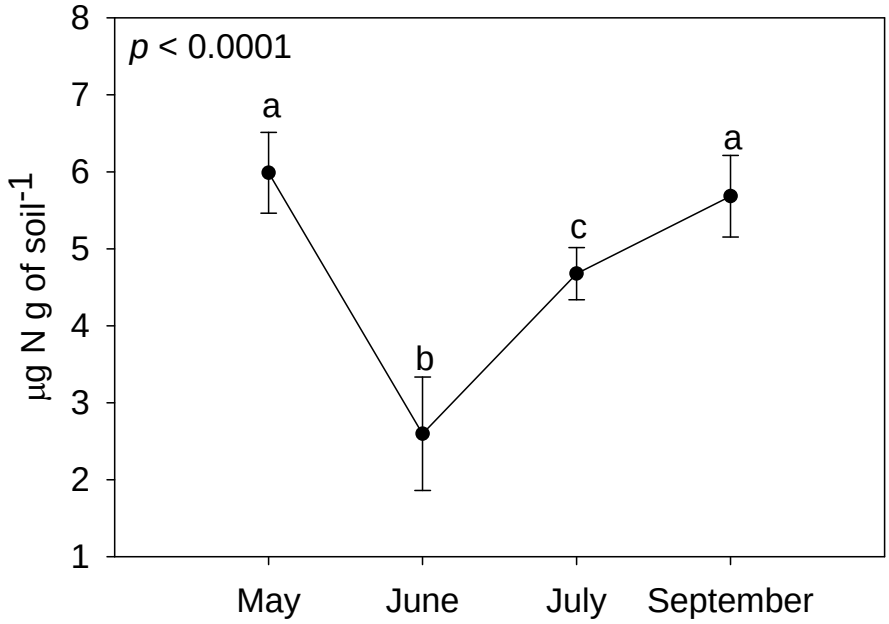


Figure 2-4. Mean (± SE) of light transmittance over 3 years. Treatments included control (C), %50 *P. virgatum* removal (PV50), 100% *P. virgatum* removal (PV100), 50% *A. gerardii* removal (AG50), and 100% *A. gerardii* removal (AG100) (n=6 for each treatment). *P*-values listed are for significant main effects for treatment (A) and sampling date (B). No significant interaction (time × treatment) occurred. Means accompanied by the same letter were not significantly different ($\alpha = 0.05$).

A.



B.

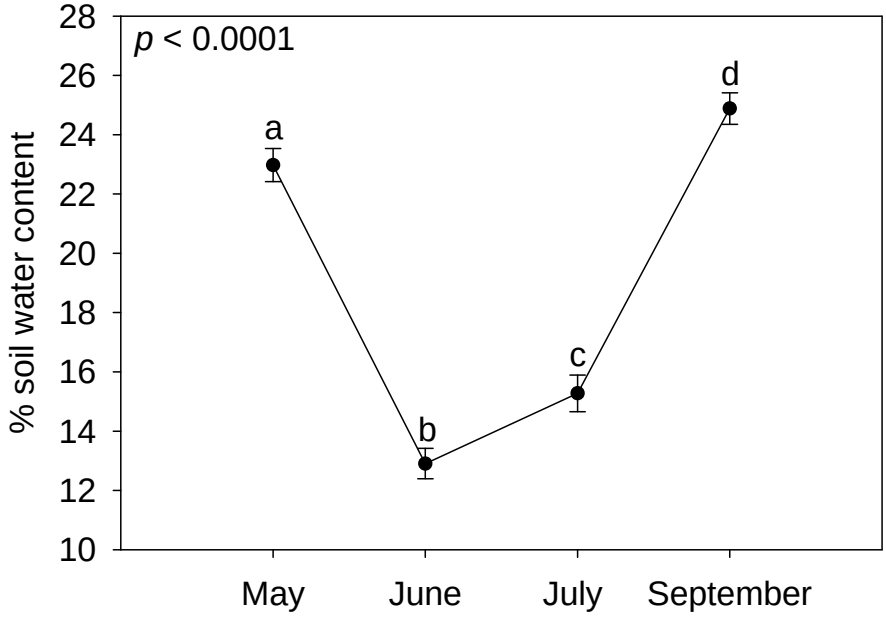


Figure 2-5. Mean (± SE) concentrations of total extractable inorganic N (A) and percent soil water content (B) across the growing season in 2006. Only the main effect of sampling date was significant. Means accompanied by the same letter were not significantly different ($\alpha = 0.05$).

Table 2-1. Means (± SE) of plant ANPP (g m^{-2}) for each year and across all years. Treatments included control, 50% *P. virgatum* removal (PV50), 100% *P. virgatum* removal (PV100), 50% *A. gerardii* removal (AG50), and 100% *A. gerardii* removal (AG100) (n=6 for each treatment). Within each year, significant differences among treatments are indicated by numbers 1-3. Significant main effects occurred for year and removal treatment; differences among treatments (across all years) are indicated by letters a-c and differences among years (over all treatments) are indicated by letters x-z. Means accompanied by the same letter or number were not significantly different ($\alpha = 0.05$)

	Year 1 (2005)	Year 2 (2006)	Year 3 (2007)	Across all years
Grass ANPP (g m^{-2})				(p<0.0001)
Control	328.87 (47.99)[1]	170.07 (24.91)[1]	302.04 (61.46)[1]	266.99 (49.08)[a]
PV50	243.99 (52.68)[1]	165.15 (41.17)[1]	265.35 (47.96)[1]	224.83 (30.47)[a]
PV100	180.98 (50.18) [2]	166.16 (28.50)[1]	352.58 (47.71)[1]	233.24 (59.82)[a]
AG50	140.67 (30.60)[23]	127.55 (24.44)[2]	114.12 (14.66)[2]	127.45 (7.67)[b]
AG100	59.77 (32.72)[3]	54.08 (23.33)[2]	101.42 (34.21)[2]	71.76 (14.92)[b]
Over all treatments (p= 0.0012)	190.86 (45.6)[y]	136.60 (22.02)[z]	227.10 (50.69)[y]	
Forb ANPP (g m^{-2})				(p = 0.0012)
Control	51.43 (38.60)[1]	29.46 (28.34)[1]	4.44 (3.56)[1]	28.44 (13.57)[a]
PV50	63.86 (26.34)[1]	22.17 (9.40)[1]	124.15 (73.25)[1,2]	70.06 (29.60)[a]
PV100	48.56 (41.15)[1]	20.15 (17.79)[1]	18.68 (15.81)[12]	29.13 (9.72)[a]
AG50	137.73 (71.55)[1,2]	22.63 (13.70)[1]	182.43 (32.07)[2]	113.60 (47.44)[ab]
AG100	268.47 (65.37)[2,3]	169.38 (76.0)[2]	417.03 (127.26)[3]	284.96 (71.97)[b]
Over all treatments (p = 0.0017)	113.61 (41.86)[y]	52.76 (29.20)[z]	149.35 (74.65)[y]	

Total ANPP (g m^{-2})				(p = 0.6481)
Control	380.30 (41.94)	223.54 (36.12)	302.04 (61.46)	306.45 (45.48)
PV50	307.85 (75.77)	205.78 (43.11)	265.35 (47.96)	309.04 (59.96)
PV100	229.54 (46.75)	201.15 (31.35)	352.58 (47.71)	273.68 (58.91)
AG50	276.40 (59.78)	172.61 (21.35)	114.12 (14.66)	264.10 (49.65)
AG100	328.24 (76.00)	237.90 (83.81)	101.42 (34.21)	371.75 (92.43)
Over all treatments (p < 0.0001)	304.47 (25.23)^{x}	208.20 (11.04)^{y}	402.35 (40.52)^{z}	

Table 2-2. Means (± SE) of percent plant cover for each year and across all years. Treatments included control, 50% *P. virgatum* removal (PV50), 100% *P. virgatum* removal (PV100), 50% *A. gerardii* removal (AG50), and 100% *A. gerardii* removal (AG100) (n=6 for each treatment). Within each year, significant differences among treatments are indicated by numbers 1-4. Significant main effects occurred for year and removal treatment; differences among treatments (across all years) are indicated by letters a-d and differences among years (over all treatments) are indicated by letters x-z. Means accompanied by the same letter or number were not significantly different ($\alpha = 0.05$).

	Year 1 (2005)	Year 2 (2006)	Year 3 (2007)	Across all years
Grass Cover				(p<0.0001)
Control	108.83 (5.04)[1]	72.5 (6.39)[1]	91.67 (11.88)[1]	91.00 (10.49)[a]
PV50	83.67 (3.70)[2]	56.33 (9.29)[2]	61.33 (8.65)[2]	67.11 (8.40)[b]
PV100	71.33 (3.78)[2,3]	49.67 (3.87)[2,3]	55.00 (5.48)[2]	58.67 (6.52)[bc]
AG50	59.67 (3.86)[3]	39.17 (3.96)[3]	61.67 (10.38)[2]	53.50 (7.19)[cd]
AG100	33.33 (2.69)[4]	23.00 (6.45)[4]	39.00 (10.55)[2]	31.78 (4.68)[d]
Over all treatments (p<0.0001)	71.37 (12.53)[x]	48.13 (8.29)[y]	61.73 (8.54)[z]	
Forb Cover				(p = 0.0128)
Control	18.33 (8.91)[1]	8.50 (5.07)[1]	10.50 (4.72)[1]	12.44 (3.00)[a]
PV50	23.00 (2.93)[1]	31.67 (9.73)[1,2]	46.67 (9.61)[2]	33.78 (6.91)[b]
PV100	24.17 (4.27)[1]	19.83 (8.16)[1,2]	27.17 (11.96)[1,2]	23.72 (2.13)[ab]
AG50	42.67 (6.81)[2]	33.83 (8.12)[2]	47.17 (15.19)[2]	41.22 (3.92)[b]
AG100	41.83 (5.47)[2]	33.67 (8.98)[2]	52.33 (5.19)[2]	42.61 (5.40)[b]
Over all treatments (p = 0.0284)	30.00 (5.10)[yz]	25.50 (4.98)[y]	36.77 (7.84)[z]	

Total Cover				(p = 0.0638)
Control	127.17 (7.35)[1]	82.67 (8.90)	102.17 (9.80)	104.00 (12.88)
PV50	106.67 (3.18)[2]	88.00 (14.63)	108.00 (15.97)	100.89 (6.46)
PV100	95.50 (5.07)[2,3]	69.50 (10.70)	82.17 (11.05)	82.39 (7.51)
AG50	102.33 (4.18)[2]	70.33 (7.26)	108.83 (10.66)	93.83 (11.90)
AG100	75.17 (4.19)[3]	59.33 (9.20)	91.33 (12.99)	75.28 (9.24)
Over all treatments (p < 0.0001)	101.37 (8.41)[y]	73.97 (5.10)[z]	98.50 (5.14)[y]	

Table 2-3. Means (± SE) of species richness (no. spp. 0.25 m^{-2}), evenness (J), and diversity (H') for each year and across all years. Community indices were calculated separately for A) the whole community, and B) the community excluding the target species removed (*Andropogon gerardii* or *Panicum virgatum*). Treatments included control, 50% *P. virgatum* removal (PV50), 100% *P. virgatum* removal (PV100), 50% *A. gerardii* removal (AG50), and 100% *A. gerardii* removal (AG100) (n=6 for each treatment). Within each year, significant differences among removal treatments are indicated by numbers 1-3. Significant main effects occurred for year and removal treatment; differences among treatments (across all years) are indicated by letters a-c, and differences among years (over all treatments) are indicated by letters y-z. Means accompanied by the same letter or number were not significantly different ($\alpha = 0.05$).

	Year 1 (2005)	Year 2 (2006)	Year 3 (2007)	Across all years
A) Whole Community				
Species Richness				($p = 0.0185$)
Control	5.67 (0.42)	4.33 (0.49)[1]	4.67 (2.97)[1]	4.89 (0.40)[a]
PV50	5.33 (0.82)	4.17 (0.31)[1]	5.17 (1.17)[1]	4.89 (0.36)[a]
PV100	5.50 (0.54)	5.00 (0.52)[1]	4.50 (1.76)[1]	5.00 (0.29)[a]
AG50	5.67 (0.76)	5.50 (0.43)[1,2]	7.33 (1.86)[2]	6.17 (0.59)[b]
AG100	6.67 (1.21)	6.50 (0.62)[2]	8.17 (2.4)[2]	6.44 (0.15)[b]
Over all treatments ($p = 0.1631$)	5.77 (0.23)	5.10 (0.42)	5.57 (0.53)	
Native prairie	…	…	8.00 (0.93)	

Species Evenness				(p = 0.0011)
Control	0.65 (0.04)[1,2]	0.56 (0.08)[1]	0.71 (0.19)[1]	0.64 (0.04)[a]
PV50	0.68 (0.04)[1]	0.69 (0.07)[1,2]	0.72 (0.10)[1]	0.70 (0.01)[a]
PV100	0.56 (0.05)[2]	0.70 (0.04)[1,2]	0.59 (0.09)[2]	0.61 (0.04)[a]
AG50	0.83 (0.02)[3]	0.85 (0.04)[2]	0.81 (0.09)[1]	0.83 (0.01)[b]
AG100	0.71 (0.04)[1]	0.59 (0.16)[1]	0.77 (0.14)[1]	0.69 (0.05)[a]
Over all treatments (p = 0.5237)	0.69 (0.04)	0.68 (0.05)	0.72 (0.04)	
Native prairie	…	…	0.89 (0.02)	
Species Diversity				(p = 0.0004)
Control	1.13 (0.10)[1,2]	0.80 (0.14)[1]	0.99 (0.44)[1]	0.97 (0.06)[a]
PV50	1.13 (0.04)[1]	0.99 (0.14)[1]	1.14 (0.18)[1]	1.09 (0.05)[a]
PV100	0.95 (0.08)[1]	1.08 (0.08)[1,2]	0.86 (0.32)[1]	0.96 (0.06)[a]
AG50	1.39 (0.07)[3]	1.45 (0.11)[2]	1.57 (0.23)[2]	1.47 (0.05)[b]
AG100	1.35 (0.12)[2,3]	1.07 (0.32)[2]	1.36 (0.42)[2]	1.26 (0.09)[b]
Over all treatments (p = 0.8187)	1.13 (0.11)	1.08 (0.11)	1.18 (0.13)	
Native prairie	…	…	1.81 (0.11)	

B) Community excluding target species [either *A. gerardii* (AG) or *P. virgatum* (PV)]

Species Richness				(p = 0.0188)
Control – PV	4.67 (0.42)	3.33 (0.42)[1]	3.33 (1.03)[1]	3.77 (0.89)[a]
PV50 – PV	4.33 (0.22)	3.17 (0.31)[1]	4.50 (1.14)[1]	4.00 (0.42)[a]
PV100 – PV	4.50 (0.22)	4.00 (0.52)[1]	4.33 (1.10)[1]	4.28 (0.19)[a]
Control – AG	4.67 (0.42)	3.33 (0.42)[1]	4.67 (1.03)[1]	4.22 (0.44)[a]
AG50 – AG	4.67 (0.42)	4.50 (0.43)[1,2]	6.33 (1.86)[2]	5.17 (0.11)[b]
AG100 – AG	5.67 (0.49)	5.50 (0.62)[2]	5.67 (1.97)[1,2]	5.61 (0.02)[b]
Over all treatments (p = 0.0155)	4.75 (0.19)[y]	3.97 (0.40)[z]	4.80 (0.56)[y]	
Native prairie	…	…	8.00 (0.93)	
Species Evenness				(p = 0.001)
Control – PV	0.51 (0.05)[1]	0.46 (0.08)[1,3]	0.65 (0.33)	0.54 (0.06)[a]
PV50 – PV	0.62 (0.04)[1,2]	0.72 (0.10)[1,2]	0.68 (0.12)	0.67 (0.03)[a]
PV100 – PV	0.57 (0.04)[1]	0.70 (0.09)[1,2]	0.58 (0.08)	0.62 (0.04)[a]
Control – AG	0.53 (0.04)[1,2]	0.58 (0.04)[1]	0.68 (0.11)	0.60 (0.04)[a]
AG50 – AG	0.71 (0.03)[2]	0.73 (0.04)[1,2]	0.71 (0.11)	0.72 (0.02)[b]
AG100 – AG	0.73 (0.04)[3]	0.57 (0.21)[1,2]	0.77 (0.26)	0.69 (0.06)[b]
Over all treatments (p = 0.1038)	0.61 (0.11)	0.63 (0.14)	0.68 (0.19)	
Native prairie	…	…	0.89 (0.02)	

Species Diversity				(p = 0.0001)
Control – PV	0.78 (0.11)[1]	0.52 (0.13)[1]	0.70 (0.56)[1]	0.67 (0.08)[a]
PV50 – PV	0.88 (0.05)[1]	0.79 (0.12)[1,2]	0.99 (0.12)[1]	0.89 (0.06)[a]
PV100 – PV	0.85 (0.06)[1]	0.88 (0.08)[2]	0.83 (0.29)[1]	0.85 (0.01)[a]
Control – AG	0.82 (0.09)[1]	0.65 (0.09)[1]	0.80 (0.38)[1]	0.76 (0.05)[a]
AG50 – AG	1.03 (0.07)[2]	1.09 (0.11)[2]	1.26 (0.27)[2]	1.13 (0.08)[b]
AG100 – AG	1.26 (0.13)[2]	0.93 (0.38)[2]	1.29 (0.35)[2]	1.16 (0.12)[b]
Over all treatments (p = 0.2401)	0.94 (0.07)	0.81 (0.08)	0.98 (0.10)	
Native prairie	…	…	1.81 (0.11)	

Table 2-4. Means (± SE) of resin-collected inorganic N (μg N bag^{-1}), soil core-extracted inorganic N (μg N g $soil^{-1}$), soil water content, and light transmission (% reaching soil surface) for each year and over all treatments (except for resin-collected total inorganic N and soil water content). Treatments included 50% *P. virgatum* removal (PV50), 100% *P. virgatum* removal (PV100), 50% *A. gerardii* removal (AG50), and 100% *A. gerardii* removal (AG100) (n=6 for each treatment). Within each year, significant differences among treatments are indicated by numbers 1-3. Significant main effects occurred for year, but not for treatment (except for light); differences among years (over all treatments) are indicated by letters x-z. For light availability differences among treatments are indicated by letters a-c. Means accompanied by the samenumber (within given year) or letter (among years) were not significantly different (α = 0.05).

	Treatment	Year 1 (2005)	Year 2 (2006)	Year 3 (2007)
Total inorganic	Control	…	…	303.67 (80.20)
N (resin)	PV50	…	…	247.00 (38.00)
	PV100	…	…	260.67 (28.63)
	AG50	…	…	204.83 (41.63)
	AG100	…	…	215.80 (49.34)
	Type III F, *p*	…	…	0.6848
Total inorganic	Control	5.62 (0.36)	5.95 (0.62)	3.23 (0.28)
N (core)	PV50	5.78 (0.42)	6.27 (0.54)	3.40 (0.21)
(September)	PV100	6.71 (0.59)	5.53 (0.42)	2.88 (0.11)
	AG50	6.77 (0.47)	5.50 (0.32)	2.79 (0.25)
	AG100	6.64 (0.44)	5.16 (0.74)	2.89 (0.28)
	Type III F, *p*	0.2484	0.6575	0.3863
	Over all treatments ($p < 0.0001$)	6.30 (0.25)^{x}	5.68 (0.19)^{y}	3.04 (0.12)^{z}
NO_3-N	Control	1.34 (0.32)	0.95 (0.11)	0.56 (0.12)
(September)	PV50	1.92 (0.28)	1.17 (0.07)	0.64 (0.11)
	PV100	1.91 (0.29)	1.00 (0.08)	0.51 (0.07)

	AG50	2.21 (0.30)	1.16 (0.07)	0.60 (0.13)
	AG100	1.92 (0.39)	1.02 (0.11)	0.55 (0.09)
	Type III F, *p*	0.2107	0.2187	0.4771
	Over all treatments ($p < 0.0001$)	1.86 (0.14)^{x}	1.06 (0.04)^{y}	0.57 (0.02)^{z}
NH_4-N	Control	4.28 (0.38)	5.00 (0.54)	2.67 (0.20)
(September)	PV50	3.86 (0.45)	5.10 (0.52)	2.76 (0.18)
	PV100	4.80 (0.53)	4.53 (0.39)	2.37 (0.13)
	AG50	4.56 (0.32)	4.34 (0.32)	2.19 (0.16)
	AG100	4.72 (0.32)	4.14 (0.71)	2.34 (0.21)
	Type III F, *p*	0.4750	0.6326	0.4307
	Over all treatments ($p < 0.0001$)	4.44 (0.17)^{y}	4.62 (0.19)^{y}	2.47 (0.11)^{z}
Soil water	Control	13.42 (0.43)	25.37 (0.43)	22.09 (0.66)
content (%)	PV50	12.48 (0.54)	24.95 (0.75)	22.05 (0.64)
(September)	PV100	12.47 (0.55)	24.60 (0.45)	21.65 (0.28)
	AG50	12.72 (0.63)	25.09 (0.45)	21.46 (0.55)
	AG100	11.32 (0.32)	24.46 (0.48)	21.80 (0.45)
	Type III F, *p*	0.0776	0.7149	0.8785
Light	Control[a]	25.18 (5.25)[1]	34.58 (3.57)	32.72 (6.91)
(July)	PV50[ab]	36.51 (9.08)[1,2]	37.58 (4.81)	34.72 (6.81)
	PV100[b]	39.39 (5.89)[1,2]	39.53 (7.65)	30.43 (6.80)
	AG50[bc]	50.30 (6.17)[2,3]	44.75 (4.36)	32.74 (5.59)
	AG100[c]	61.88 (7.52)[3]	54.11 (7.42)	42.17 (9.08)
	Type III F, *p*	0.0140	0.1800	0.8049
	Over all treatments ($p < 0.0001$)	42.65 (6.25)^{y}	42.11 (3.43)^{y}	34.56 (2.02)^{z}

Appendix 1. Original species and seeding rates used in a prairie restoration initiated in 1998. Nomenclature follows USDA, NRCS Plants Database (2007).

Dominant grasses (160 seeds m^{-2})	Common species (16 seeds m^{-2})
Andropogon gerardii	*Artemisia ludoviciana*
Panicum virgatum	*Bouteloua curtipendula*
Schizachyrium scoparium	*Salvia azurea*
Sorghastrum nutans	*Solidago canadensis*
	Symphyotrichum ericoides

Frequent species (10 seeds m^{-2})	Uncommon species (5 seeds m^{-2})
Amorpha canescens	*Asclepias viridis*
Asclepias verticillata	*Baptisia australis*
Brickellia eupatorioides	*Baptisia bracteata*
Ceanothus herbaceus	*Callirhoe involucrata*
Dalea purpurea	*Dalea candida*
Koeleria macrantha	*Desmanthus illoenisis*
Lespedeza capitata	*Echinacea angustifolia*
Mimosa nuttallii	*Liastris punctata*
Solidago missouriensis	*Lomatium foeniculaceum*
Sporobolus compositus	*Oenothera macrocarpa*
Sporobolus heterolepis	*Packera plattensis*
Symphyotrichum oblongifolium	*Penstemon cobaea*
Vernonia fasciculata	*Penstemon grandiflorus*
	Psoralidium tenuiflorum
	Ratibida columnifera
	Rosa arkansana
	Ruellia humilis
	Symphyotrichum sericeum
	Sisyrinchium campestre

CHAPTER 3 - PLANT PRODUCTIVITY AND COMMUNITY RESPONSES TO SOIL RESOURCE MANIPULATION AND ABOVEGROUND BIOMASS REMOVAL IN RESTORED GRASSLAND

Abstract

The outcome of tallgrass prairie restorations is often the successful establishment of the dominant C_4 grasses, while many forb species are more difficult to establish and slow to increase in cover and abundance leading to low overall diversity in these communities. In order to enhance the diversity of restored grasslands, it may be necessary to design management strategies that alter resource availability (e.g. carbon additions and mowing) to reduce the dominance of C_4 grasses and promote forb diversity. In this study we used long-term manipulations of soil nutrient availability (ambient, reduced-, and enriched-N treatments) and soil depth (shallow vs. deep soil) established in 1998, with the addition of a mowing treatment in 2005, to assess the independent and interactive effects of these manipulations on plant productivity and community responses in a restored tallgrass prairie.

In the altered nutrient availability treatments, carbon amendments in the form of sucrose reduced soil N availability in both mowed and unmowed plots, but no treatment combination significantly increased forb productivity or cover, and mowing actually decreased forb productivity. The N-fertilizer addition resulted in greater cover and productivity of the dominant grass, *Andropogon gerardii.* In the altered soil depth treatment, shallow soils resulted in lower grass productivity and lower total ANPP, but did not increase forb productivity. Overall, species diversity and richness were not affected during the three years of this study by manipulations of soil nutrient availability, soil depth, or mowing, which may be related to the high cover of *A. gerardii* across all treatments. This suggests that manipulation of soil resources (N availability or soil depth) may not be sufficient to inhibit the dominance of well-established C_4 grasses, at least within the time frame of this study. Longer-term measurements may be required to detect

significant increases in forb productivity and cover, or further management may be required in order to enhance plant species diversity and richness in this grass-dominated prairie restoration.

Key Words: C amendment, grassland restoration, mowing, plant species diversity, soil nutrient availability

Introduction

Prior to European settlement, the North American tallgrass prairie covered more than 68,000,000 ha of the Great Plains (Samson and Knopf 1994; Robertson et al. 1997), but more than 96% of the pre-European extent of native tallgrass prairie has been lost due to conversion to agriculture, fragmentation, exotic species invasion and fire suppression (Samson and Knopf 1994). The decline of tallgrass prairie ecosystems accentuates the need to develop better methods to restore community composition and ecosystem services (Webb 1996). One common restoration practice is the reintroduction of native prairie species into previously disturbed soils. However, establishment of grass and forb species is generally uneven, and plant species diversity is often slow to recover even with efforts to sow additional forb species (Warkins and Howell 1983; Sperry 1994) or with adjacent native vegetation as a source for colonization (Kindscher and Tieszen 1998). Understanding the mechanisms that regulate productivity and species composition in native prairie may provide insights useful for accelerating the recovery of diversity in restored communities.

Native tallgrass prairie composition and productivity are influenced by several factors, including fire, grazing, topography, and climate (Abrams and Hulbert 1987; Knapp et al. 1993; Briggs and Knapp 1995; Howe 1995, 1999; Hartnett et al. 1996; Knapp et al. 1998). These factors and their interactions affect the availability of several key resources: light, water, and nitrogen (Knapp and Seastedt 1986; Schimel et al. 1991). Many studies conducted in native tallgrass prairie have shown that plant species diversity and productivity are affected by N availability (influenced by topography and fire), light availability (affected by grazing/mowing and fire) and soil depth (associated with topography). For example, experimentally enhanced N availability increases productivity of the dominant grasses, resulting in decreased diversity (Wilson and Tilman 1991; Collins et al. 1998; Piper et al. 2005; Clark et al. 2007; Clark and Tilman 2008). Additionally, light availability appears to be the major mechanism promoting

higher species diversity in grazed vs. ungrazed prairie, and in maintaining species richness in annually burned N-amended plots, where species richness declined in the absence of mowing (Collins et al. 1998). Furthermore, deep lowland prairie soils are more productive than shallow upland soils (Briggs and Knapp 1995), but shallow upland soils have greater plant species diversity presumably due to the shallow rooting depth negatively affecting the dominant grasses resulting in a competitive release for subordinate forb species (Abrams and Hulbert 1987; Gibson and Hulbert 1987; Collins 1992).

Interactions among N availability, light availability and soil depth are also important during tallgrass prairie restoration and understanding their relationships may be the key to successful species-rich restorations (Howe 1999). A long-term restoration experiment at Konza Prairie Biological Station, established in 1998, has been used to assess how manipulation of soil depth and N availability affect plant species diversity and productivity (Baer et al. 2003). Soil depth had no effect on productivity or diversity after 3 years, but Baer and others (2003) found that the enriched-N treatment (N fertilizer addition) had the highest productivity and lowest diversity, while the reduced-N treatment (sawdust-amended soils to promote N immobilization) had the lowest productivity and highest diversity. Sucrose has also been used as a carbon source to reduce N availability in shrublands, alpine soils, shortgrass steppe, temperate grassy woodland, and grassland restorations (McLendon and Redente 1992; Jonasson et al. 1996; Paschke et al. 2000; Prober et al. 2005; Szili-Kovács et al. 2007). Reduced N availability in grasslands has been reported to increase species richness and diversity (McLendon and Redente 1992; Paschke et al. 2000). Mowing also has been used during restoration to decrease the cover of the dominant vegetation, allowing for subordinate species to increase through increased light and space availability (Maron and Jefferies 2001; Tix and Charvat 2005).

In this study, we continued a long-term investigation into the relationships among N availability, soil depth, plant productivity, and species diversity in an ongoing restoration experiment at the Konza Prairie Biological Station. In addition to assessing long-term responses to the soil depth and N availability treatments, a new mowing treatment was added to investigate the effect of aboveground biomass removal and subsequent increases in light availability in promoting species diversity during restoration. Experimental manipulations included two levels of soil depth, three levels of N availability, and a mowing treatment, all implemented in a long-term prairie restoration experiment initiated in 1998 in a former agricultural field. Our first

objective was to evaluate the effectiveness of soluble carbon amendments (sucrose) as a means of maintaining reduced N availability in the treatment originally established by sawdust additions. In addition to this methodological objective, we had two additional objectives: (1) to examine the interactive effects of aboveground biomass removal via mowing and the soil depth or N availability treatments on plant productivity and composition; and (2) to assess the relationships between resource availability, productivity, and diversity under conditions of altered soil N availability, altered soil depth, and with or without aboveground biomass removal. We hypothesized that mowing would interact with low soil N or shallow soils to decrease grass productivity and increase plant diversity; while lack of mowing plus N-fertilizer or deep soils would promote the greatest grass productivity leading to decreased diversity. These hypotheses were based on the general inverse productivity-diversity relationship (regulated by N availability, light availability and soil depth) observed in native tallgrass prairie, as well as earlier results from this restoration experiment. We also hypothesized that altering levels of N availability (reduced-, ambient-, and enriched-N), soil depth (deep and shallow), and light availability (mowed and unmowed) would lead to productivity and diversity gradients that would allow comparisons of the relationships among resource availability, productivity, and diversity in restored tallgrass prairie, similar to relationships reported for native tallgrass prairie.

Methods

Study site

Research plots used here were part of a prairie restoration experiment established in 1998 (see Baer et al. 2003) in a former lowland agricultural field at Konza Prairie Biological Station (KPBS), a 3487 ha tallgrass prairie preserve located in the Flint Hills region of Northeastern Kansas (39°5´N, 96°35´W). Mean annual precipitation at the site is 834 mm yr^{-1} (1891-2002) with high variability between years (coefficient of variation = 24%); approximately 635 mm falls during the growing season (April through September) of each year (Sophocleous 1998). In the three years of study (2005, 2006, 2007) total precipitation was 959, 631, and 693 mm, of which 707, 570, and 607 mm fell during the growing season (April through September) of each year, respectively. Even though total rainfall in 2006 was near average, 301 mm occurred late in the growing season (August through September) and water stress was evident through much of the growing season. Prior to restoration, the site had been cultivated for more than 50 years. The

soil was a Reading silt loam (mesic Typic Argiudoll) formed by alluvial and colluvial deposits. Following initiation of the restoration experiment, the area became dominated by a few C_4 grass species, predominantly *Andropogon gerardii* Vitman and *Panicum virgatum* L., with *Sorghastrum nutans* (L.) Nash being relatively common (nomenclature follows USDA, NRCS Plants Database [2007]). The remaining plant community consisted primarily of a few forb species, including *Salvia azurea* Michx. ex Lam., *Baptisia australis* (L.) R.Br. ex Ait. f. var. *minor* (Lehm.) Fern., *Lespedeza capitata* Michx., *Brickellia eupatorioides* (L.) Shinners, and *Vernonia fasciculata* Michx. The entire restored field, including the experimental plots, was burned frequently with prescribed spring fires

Establishment of experimental plots

In June 1997, 16 whole plots were delineated in a 3.2 ha agricultural field at KPBS. Replicated blocks of four 6 × 8 m whole plot treatments with four combinations of varying soil depth and nutrient availability manipulations were established, with each whole plot subdivided into twelve 2 × 2 m subplots for sampling (n = 192). The four whole plot treatments were assigned randomly within each block. Thus, each treatment was replicated four times in a blocked experimental design (N=4). In each block, one whole plot was assigned to an altered soil depth treatment, one whole plot was assigned to an altered nutrient availability treatment, one whole plot was assigned to a combined altered soil depth and nutrient availability treatment, and one plot was left as an untreated control (Figure 3-1). For a detailed description of the whole plot treatments and site preparation see Baer and others (2003). For the present study, only the whole plots with altered nutrient availability (reduced-, ambient- and enriched-N soil) treatments and altered soil depth (deep and shallow soil) treatments were used (Figure 3-1). Soil depth was manipulated prior to the initiation of the experiment in 1997 by excavating the soil and burying limestone slabs at approximately 25 cm deep in 2 m wide strips to produce alternating strips of deep and shallow soil. Prior to planting, sawdust was added as a recalcitrant carbon source to 2m strips within the nutrient manipulation plots and tilled into the soil to promote soil N immobilization and produce the reduced-N conditions in the altered nutrient availability treatment. Over time, this carbon source was decomposed by the soil microbial community, resulting in a decrease in N immobilization potential (Baer et al. 2003). Therefore starting in 2005, sucrose was applied as an additional labile carbon source to promote N immobilization.

Sucrose was applied at a rate of 200 g m^{-2} four times per year throughout the growing season (May to August) on the reduced-N subplots. For the enriched-N subplots, an annual application of ammonium-nitrate fertilizer equivalent to 5.0 g N m^{-2} occurred early in the growing season (early to mid June) each year since 1998.

Beginning in 2005, half of each whole plot was randomly assigned to a mowing treatment (Figure 3-1). Mowing occurred early in the growing season after the fertilizer treatment (late June 2005 and 2006, and early June 2007). A weed whacker was used to mow the plots to a height of approximately 15 to 20 cm. The mowed plant material was not removed from the plots.

Plant community establishment

In April 1998, seeds of 42 native prairie species were sown into all whole plots with varying seeding rates (dominant grasses, common, frequent, or uncommon forb species) (Appendix 1). See Baer and others (1999, 2003) for further detail on plant community establishment.

In April 2005, all whole plots were sown with a different forb species mixture than the 1998 species mix, to allow us to distinguish between forb species recruited from the initial seeding in 1998 and the second seeding in 2005. All forb species added in 2005 were sown at a rate of 25 seeds m^{-2} (Table 3-1). Prior to planting, all whole plots were lightly raked. The seeds were mixed with builder's sand and hand broadcast evenly over the whole plots.

Belowground sampling

Soils were sampled for extractable inorganic N (NO_3^- and NH_4^+) in midseason (mid July) and late season (late September) in 2005, 2006 and 2007. Two soil cores (10 cm deep x 2 cm diameter) were taken from each 2 × 2 m subplot, composited, crumbled by hand and sieved through 4-mm mesh to remove roots and rocks and stored at 4°C. In 2007, the altered nutrient availability plots were sampled to a depth of 20 cm and cores were divided into 0-10 cm and 10-20 cm subsamples. Inorganic N was extracted from 11-12 g field moist subsamples using 2 $mol \cdot L^{-1}$ KCl, and filtered through 0.4-µm polycarbonate filters (Osmonics Inc.). Extracts were analyzed colorimetrically for NO_3-N and NH_4-N on an Alpkem Flow Solution® autoanalyzer (OI analytical, College Station, Texas, USA). Nitrate (NO_3-N) was determined by diazotization with sulfanilamide after reduction through a cadmium coil, and ammonium (NH_4-N) was

measured using the phenol blue method (Keeney and Nelson 1982). The remaining soil was weighed field moist, dried for 2 days at 60°C, and reweighed to determine gravimetric soil water content.

In 2005 and 2007, ion exchange resin bags were buried in each subplot to provide another index of relative inorganic N availability (Binkley and Hart 1989). Bags were constructed of nylon mesh material and filled with a 1:1 mixture (10 g total) of cation exchange resin (Dowex HCR-W2) and anion exchange resin (Dowex 1 X 8-50) pre-loaded with H^+ and Cl^-, respectively. One resin bag was buried in the surface 10 cm in each subplot in late May and harvested in September. In the laboratory, resin bags were rinsed under running deionized water to remove excess soil, and extracted with 100 mL of 2 mol·L^{-1} KCl by shaking for 2 hours at 200 rpm. Extracts were then filtered and analyzed using the Alpkem Flow Solution® autoanalyzer (OI analytical, College Station, Texas, USA) as described above. Since the resin bag extracts were acidic, all samples were neutralized prior to analysis.

Aboveground sampling

In 2007, light transmission through the plant canopy was quantified in all subplots before (3 June) and after (8 July) mowing (plots were mowed on 8 June). All measurements were made at midday (1100-1300, Central Daylight Time) under full sun conditions. Three measurements of photosynthetic photon flux density (PPFD) (μmol·m^{-2}·s^{-1}) were taken in each subplot. One measurement was made above the plant canopy and two orthogonal measurements were made at the soil surface with a 0.5 m Sunfleck ceptometer (Decagon, Pullman, Washington). The two soil surface measurements were averaged for each subplot, and light transmission was expressed as percent PPFD reaching the soil surface.

In all years, percent cover of each plant species was visually assessed in spring (late May-early June) and summer (August) for all plants rooted within a 0.25-m^2 quadrat in each subplot. For each species, the maximum cover value from the combined spring and summer sample dates in a given year was used to calculate plant species richness, diversity, and evenness. Species richness (S) was calculated as the number of plant species per 0.25-m^2 quadrat. Diversity was calculated for each subplot using Shannon's diversity index, $H' = -\Sigma p_i \ln p_i$, where p_i represented the proportion of total cover contributed by species i. Shannon's diversity index was selected because it includes proportional representation of species in a community and provides relatively

even weighting to both richness and evenness (Barbour et al. 1999). Evenness was calculated using Pielou's index, $J = H'/H_{max}$, where H_{max} represented the natural log of S.

Towards the end of each growing season (late August, early September), aboveground biomass was harvested from each subplot from an area outside the species composition sampling quadrat. Vegetation from one 0.10-m^2 area in each plot was clipped at ground level and sorted into plant biomass categories as follows. In 2005, the categories included live grass, live forb, dead grass, dead forb, and unidentified surface plant litter. In 2005, live and dead grass and live and dead forb biomass was summed to estimate annual grass and forb productivity, respectively, and combined grass and forb biomass + litter was summed to estimate total aboveground net primary productivity (ANPP), a measure of ecosystem function (Briggs and Knapp 1995). In 2006 and 2007, the categories included only standing grass mass, standing forb mass, and unseparated surface plant litter. In June of 2006 and 2007, pre-mowing (June) plant biomass was harvested from the mowed subplots by clipping to the mean height of mowing. The June biomass was added to the end-of-season biomass to determine total biomass produced over the growing season for the mowed subplots (n=48). In all cases, biomass was oven-dried at 60°C for at least 48 hours prior to weighing. Annual productivity estimates represented only biomass produced in the year of measurement, since all treatment plots were annually burned in spring during the period of study.

Statistical analyses

The altered soil depth plots and altered nutrient availability plots were analyzed separately due to differing experimental designs. Resource availability (soil moisture, N and light), plant cover, productivity, and community indices (species richness, evenness and diversity) were analyzed according to a split block design for the altered soil depth experiment and a strip design for the altered nutrient availability experiment. Data on core-collected N availability, plant productivity, plant cover, and community indices were analyzed by repeated measures analysis. Soil moisture was not analyzed with repeated measures because soil water content varies with recent precipitation events. Resin-collected N was not analyzed with repeated measures analysis since 2006 data were not collected. All data were analyzed using mixed-model analysis of variance (SAS Version 9.1; SAS Institute, Inc. 2002-2003). Denominator degrees of freedom were estimated using the Satterthwaite's method for all tests of

fixed effects (mow, stone and mow × stone for the altered soil depth experiment and mow, nutrient and mow × nutrient for the altered nutrient availability experiment). All means comparisons were performed using the difference in least squares means procedure, $\alpha = 0.05$ (SAS 2002-2003). Relationships between response variables were examined using correlation analyses (r = Pearson correlation coefficient) in SAS, $\alpha = 0.05$.

Results

Resource availability

Resin-collected inorganic N

The altered nutrient availability treatments influenced relative soil N availability, as indicated by resin-collected inorganic N. Resin-collected inorganic N was significantly greater in the enriched-N treatment compared to other treatments in both years sampled, with differences among treatments driven largely by differences in NO_3-N (Table 3-2A). In 2005, there were main effects of the nutrient treatments for resin-collected total inorganic N ($p = 0.0024$), NO_3-N ($p = 0.0025$), and NH_4-N ($p = 0.0295$), and resin-collected N for all categories was greater in the enriched-N treatments compared to the reduced-N and ambient treatments (Table 3-2A). In 2005, total inorganic N in the enriched-N treatment was ~4× greater and NO_3-N was ~5× greater than under ambient conditions ($p = 0.0024$). In 2005, the enriched-N treatment also had higher resin-collected NH_4-N ($p = 0.0295$), but in 2007 there were no differences in resin-collected NH_4-N among treatments ($p = 0.2713$; Table 3-2A). In 2005, the reduced-N treatment had ~70% less resin-collected total inorganic N, 94% less resin-collected NO_3-N, and ~20% less resin-collected NH_4-N compared to ambient conditions, but these values were not significantly different. In 2007, there were main effects of the nutrient treatments for resin-collected total inorganic N ($p = 0.0210$), and NO_3-N ($p = 0.0097$), but not for NH_4-N ($p = 0.2713$). Again, resin-collected total N and NO_3-N were greater in the enriched-N treatments compared to reduced-N and ambient treatments (Table 3-2A). Nitrate has greater mobility in the soil compared to ammonium; therefore, resin-collected NO_3-N may be a better indicator of differences in growing season plant available nitrogen compared to resin-collected NH_4-N (Binkley 1984). In 2007, the reduced-N treatments had ~60% less resin-collected total inorganic N, ~80% less resin-collected NO_3-N, and ~25% more resin-collected NH_4-N compared to

ambient conditions, and these values were not different from ambient conditions (Table 3-2A). Resin-collected inorganic N did not vary with the soil depth treatments (Table 3-2B) or with mowing treatments (data not presented).

Extractable inorganic nitrogen

In all three years, soil cores were collected mid-season (July) and at the end of the season (September) to determine concentrations of extractable inorganic N. Mid-season soil cores from the altered nutrient availability plots revealed a main effect of nutrient treatment on total extractable inorganic N for 2005 ($p = 0.0506$) and 2006 ($p = 0.0290$), but not for 2007 ($p = 0.0974$) (Table 3-3A). Across all years, the enriched-N treatment had greater concentrations of total extractable inorganic N ($p = 0.0074$; Table 3-3A), relative to the reduced-N or ambient treatments. Total extractable inorganic N was reduced by the sucrose addition each year, but the reduction was not significant (Table 3-3A). Concentrations of total extractable N also varied across years, with all three years being significantly different from one another when averaged across all nutrient treatments (Table 3-3A). Concentrations of extractable NH_4-N did not vary with treatment within year or across all years (Table 3-3A). For extractable NO_3-N, there was a main effect of year in the nutrient availability treatments ($p < 0.0001$), with all years being different from one another (Table 3-3A). There was no effect of mowing in the nutrient availability plots, and no interaction between mowing and nutrient treatments. The 10-20 cm soil cores did not lead to differing results among the treatments.

Mid-season total extractable N was not affected by the altered soil depth treatments within or across years, but total extractable N was different among all years ($p < 0.0001$), with all years being different from one another (Table 3-3B). The relative differences among years were consistent in both altered nutrient availability and altered soil depth plots, likely reflecting interannual differences in climate. There was a main effect of the mowing treatment across all years ($p = 0.0340$), with mowing resulting in greater total extractable inorganic N compared to unmowed subplots (Table 3-3B). NH_4-N did not vary with soil depth treatment or with mowing treatment (Table 3-3B). NO_3-N did not vary with soil depth in 2005 ($p = 0.4879$) or 2006 ($p = 0.3260$), but it did vary with soil depth in 2007 ($p = 0.0208$; Table 3-3B) when NO_3-N concentration was significantly less in shallows soils (1.56 μg N g soil^{-1}) compared to deep soils (1.85 μg N g soil^{-1}). The mowing treatment increased soil NO_3-N in 2006 ($p = 0.0291$), and

averaged across all years mowing resulted in greater NO_3-N concentrations ($p = 0.0021$) compared to the unmowed treatment (Table 3-3B).

At the end of the growing season (September), differences in extractable inorganic soil N among the altered nutrient availability and soil depth treatments were not as pronounced (Table 3-4). In the altered nutrient availability treatments, there were main effects of nutrient treatments on total extractable N and NO_3-N (Table 3-4A), but no significant effect of mowing or any interaction between mowing and nutrient treatments occurred. Across all years, the reduced-N treatment had significantly lower concentrations of total extractable inorganic N ($p = 0.0336$) and NO_3-N ($p = 0.0087$; Table 3-4A). Concentrations of total extractable N also varied across years ($p < 0.0001$), with 2005 having less extractable soil nitrogen compared to other years (Table 3-4A). Concentrations of extractable NH_4-N did not vary with treatment within year or across all years (Table 3-4A). Concentrations of extractable NO_3-N also varied across years ($p = 0.0033$), with all three years being different from one another (Table 3-4A).

For the soil depth plots, there was no effect of soil depth treatments on late-season total inorganic N or extractable NH_4-N. However, concentrations of extractable NO_3-N did vary when soil depth treatments were averaged across all years ($p = 0.0182$), with shallow soils having less NO_3-N compared to deep soils (Table 3-4B). No significant main effect of mowing occurred in the soil depth treatments in the fall soil sampling.

Soil water content

Mid-season soil water content (July) did not vary with the altered nutrient availability treatments except in 2006, when the reduced-N treatment was wetter than the enriched-N treatment ($p = 0.0200$) and mowed areas were wetter than unmowed areas ($p = 0.0396$) (Table 3-2A). Soil moisture was greater in deep soils compared to shallow soils in 2006 ($p = 0.0037$) and 2007 ($p = 0.0022$) (Table 3-2B). There was no effect of mowing on soil water content in the altered soil depth plots. Although a single sampling for soil moisture cannot capture seasonal patterns, it does offer a snapshot in time of differences when samples were collected.

Light

Prior to mowing in June 2007, there were no significant main effects of nutrient treatments, soil depth treatments, or mowing conducted in previous years on light availability measured at the soil surface (data not shown). However, there was a significant mowing × soil

depth interaction (F = 5.75, p = 0.0215), with significant differences in light availability between deep and shallow soils only in areas that had never been mowed, where shallow soils had greater light availability at the soil surface (Figure 3-2). In contrast, July light availability (1 month post-mowing) was influenced by significant main effects of both altered nutrient availability and altered soil depth, as well as a significant mowing effect in both treatments (Figure 3-3). In the altered nutrient availability plots, mowed subplots had greater July light availability compared to unmowed subplots (p = 0.0087; Figure 3-3A), and the reduced-N plots had greater post-mowing (July) light availability compared to enriched-N plots (p = 0.0041; Figure 3-3C). There was no significant interaction between mowing and nutrient treatments. In the soil depth treatment, mowed subplots had greater light availability compared to unmowed subplots (p = 0.0056; Figure 3-3B), and shallow soils had greater post-mowing (July) light availability compared to deep soils (p = 0.0160; Figure 3-3D). There was no significant interaction between mowing and soil depth.

Primary productivity

Due to differences in sampling (see Methods), total ANPP was calculated including all material (forb, grass, and litter) as well as with surface litter material excluded. For all analyses, the results were similar; therefore, the following responses are based on excluding litter from the ANPP calculations to prevent overestimating total ANPP by double counting litter material (i.e. problem of distinguishing mowed material vs. litter falling naturally from the canopy), except for 2005. For 2005, total ANPP included the litter material, since there was no pre-mowing biomass collection; therefore including litter is a more suitable estimate of total ANPP in 2005.

In 2005 and averaged across all years, there was a significant main effect of the altered nutrient availability treatments on grass productivity, with the enriched-N treatment producing greater grass biomass compared to ambient or reduced-N availability treatments (Table 3-5A); however, there was no significant main effect of nutrient treatments on forb productivity (data not shown). There was a main effect of mowing across all years for forb productivity (p = 0.0440), with mowing reducing forb productivity every year of the study (Table 3-5A). Within a given year, total ANPP did not vary among nutrient treatments; however, averaged across all years, total ANPP was greatest in the enriched-N treatment (p = 0.0079; Table 3-5A). Averaged across all treatments in the altered nutrient availability plots, 2007 produced greater grass

biomass (p = 0.0005), forb biomass (p = 0.0201), and total ANPP (p = 0.0005), compared to 2005 and 2006 (Table 3-5A).

A 2-way interaction of mowing and year affected grass productivity (p = 0.0296) in the altered nutrient treatments, but this may have been an artifact of the way biomass was harvested in 2005, which excluded an estimate of pre-mowing biomass (see Methods) that was included in 2006 and 2007. When 2005 data were omitted the 2-way interaction was not significant (Figure 3-4). In the mowed treatment, the differences observed between 2006 and 2007 in grass productivity were most likely linked to differences in precipitation, since 2006 was a dry year and 2007 was a relatively wet year (Figure 3-4). In the unmowed treatment, differences among years occurred as well, with 2006 having less grass productivity compared to 2005 and 2007 which again is most likely tied to differences in precipitation (Figure 3-4).

In the altered soil depth treatments, there were no significant treatment effects within years except in 2007, when total ANPP was greater in deep soil (Table3-5B). Across all years, there was no significant main effect of soil depth (F = 3.05, p = 0.1169) or mowing (F = 2.77, p = 0.1326), but there was a significant main effect of year (F = 19.00, p <0.0001) with total ANPP in 2006 being significantly less than in 2005 and 2007 (Table 3-5B). A significant 2-way interaction occurred between mowing and year for total ANPP (p = 0.0432), but this may be an artifact of the way biomass was harvested in 2005, which excluded an estimate of pre-mowing biomass (see Methods) that was included in 2006 and 2007. When 2005 data were omitted the 2-way interaction was not significant (Figure 3-5). Differences among years occurred within both the mowed and unmowed treatments for total ANPP, with 2006 having significantly lower ANPP than other years (Figure 3-5).

Relationships between productivity and plant resources

There was a negative correlation between total ANPP and July light availability (r = -0.4122, p < 0.0001) with increased total ANPP leading to reduced light availability (Figure 3-6 top panel). The enriched-N treatment (mowed and unmowed) had the greatest total ANPP and least light reaching the soil surface (Figure 3-6 top panel). There was also a positive correlation between total ANPP and resin-collected total inorganic N (r = 0.2726, p = 0.0138) with the enriched-N treatment (mowed and unmowed) having the greatest total ANPP and total inorganic N, but this relationship was driven solely by the inclusion of the enriched-N plots (Figure 3-7

bottom panel). When the enriched-N plots were excluded there was no relationship between total ANPP and resin-collected total inorganic N among the other treatments.

Percent plant cover

In the altered nutrient availability plots, there were main effects of nutrient treatments ($p = 0.0191$) and mowing ($p < 0.0001$) on % grass cover, but there were no significant interactions between nutrient treatment and mowing (Table 3-6A). The enriched-N plots had greater grass cover compared to the other treatments both within and across years ($p = 0.0191$; Table 3-6A). Unmowed plots had greater grass cover compared to mowed plots in 2005 and 2006 and across all years (Table 3-6A). Averaged across all nutrient treatments or mowing treatments, 2007 had greater grass cover than 2005 and 2006 ($p < 0.0001$; Table 3-6A).

In the altered nutrient availability plots, significant 2-way interactions occurred between mowing and year ($p < 0.0001$), and between nutrient treatment and year ($p = 0.0148$). In the unmowed treatment, grass cover did not vary, but within the mowed treatment there was a trend for increasing grass cover over time, resulting in 2007 having the greatest grass cover (Figure 3-4). In addition, in 2005 the unmowed treatment had significantly greater grass cover than the mowed treatment, but over time the treatments converged and were similar in grass cover by 2007 (Figure 3-4). For the nutrient by year interaction, differences among years occurred within a given treatment, with 2007 having significantly greater grass cover in all nutrient treatments (Figure 3-8). In 2005, all nutrient treatments were similar, but the treatments diverged with time resulting in enriched-N treatments having the greatest grass cover by 2007 (Figure 3-8).

There were no differences among nutrient treatments for percent forb cover within a given year, but in 2006 there were significant differences between mowed and unmowed treatments ($p = 0.0784$; Table 3-6B), with the unmowed treatment having greater percent forb cover. Averaged across all treatments, 2007 had greater percent forb cover compared to previous years ($p < 0.0001$; Table 3-6B), and a significant 2-way interaction occurred between mowing and year ($p = 0.0180$). In the unmowed treatment, forb cover tended to increase over time with 2007 having the greatest percent forb cover (Figure 3-4). In the mowed treatment, 2006 had significantly less forb cover compared to other years (Figure 3-4). In addition, in 2005, the mowed and unmowed treatments were similar, but the treatments diverged over time with the

unmowed treatments having significantly greater forb cover in 2006 and 2007 compared to the mowed treatment (Figure 3-4; Table 3-6A).

There were no differences in total plant cover in response to the altered nutrient availability treatments, but there was a main effect of mowing ($p = 0.0005$). In all years, the unmowed treatment had greater total plant cover compared to the mowed treatment (Table 3-6A). In both the unmowed and mowed treatments, total plant cover in the first two years was similar, but in 2007 total plant cover was greater than in previous years ($p < 0.0001$; Figure 3-4).

In the altered soil depth plots, there was a significant main effect of mowing on grass cover and total plant cover, but not forb cover (Table 3-6B). The unmowed treatment had greater grass cover ($p = 0.0238$), forb cover ($p = 0.2397$) and total plant cover ($p = 0.0003$) compared to the mowed treatment (Table 3-6B). When compared across all treatments, 2007 had greater grass cover ($p < 0.0001$), forb cover ($p < 0.0001$), and total plant cover ($p < 0.0001$) relative to 2005 and 2006 (Table 3-6B). A significant 2-way interaction between mowing and year affected percent grass cover ($p = 0.0004$). In both mowed and unmowed treatments, 2007 grass cover was greater than earlier years (Figure 3-5). In addition, in 2005 the unmowed treatment had greater grass cover than the mowed treatment, but over time the treatments converged and were similar in grass cover by 2007 (Figure 3-5).

Relationships with dominant grass cover

The percent cover of the dominant grass (*Andropogon gerardii*) and mean total ANPP were positively correlated (Figure 3-8). As the cover of *A. gerardii* increased so did total ANPP, with the 2007 enriched-N treatment having the greatest *A. gerardii* cover and total ANPP compared to other treatments (Figure 3-9). There was also a positive correlation between percent cover of *A. gerardii* and mean diversity (averaged across mowing treatment; values from Table 3-7), which was contrary to our prediction. We predicted that as the cover of the dominant grass decreased, species diversity would increase. Our results show just the opposite with a positive trend for increasing *A. gerardii* cover to be correlated with increased diversity across nutrient and soil depth treatments (Figure 3-8). In addition, 2007 had the highest values for both percent cover of *A. gerardii* and diversity across all altered nutrient and altered soil depth treatments compared to other years (Figure 3-8, Tables 3-6, 3-7).

Community indices

In the altered nutrient availability plots, there was no main effect of mowing for species richness, species evenness, or species diversity (Table 3-7A). Within each year, neither species richness nor species diversity was affected by nutrient treatment. However, species evenness in 2007 was lower in enriched-N treatments ($p = 0.0367$). Main effects occurred for year, with 2007 having the highest species richness ($p < 0.0001$), species evenness ($p < 0.0001$) and species diversity ($p < 0.0001$; Table 3-7A). Even though the community indices showed an increasing trend with time, these values were still much lower than native prairie on comparable soil type and topography (Table 3-7A). Of the community indices calculated in this study, species evenness was most similar to the values observed in native prairie.

For the altered soil depth plots, there was no main effect of mowing on species richness, species evenness, or species diversity (Table 3-7B). Within each year, species richness, species evenness, and species diversity were slightly higher in shallow soils compared to deep soils, but only species diversity in 2006 ($p = 0.0478$) was significantly higher in shallow soil compared to deep soil (Table 3-7B). Overall, the community indices showed an increasing trend with time, but again these values are lower than native prairie with species evenness being the most similar to native prairie.

Discussion

Many restoration efforts suffer from low diversity relative to native grasslands; therefore, understanding the factors that limit or promote plant diversity in restored tallgrass prairies is required in order to design management strategies that can increase diversity. Native prairie plant communities are influenced by both abiotic (topography, fire, soil type, light, and N availability) and biotic (plant species, mycorrhizae, grazing, etc.) factors (Collins et al. 1998; Burke et al. 2002; Knapp et al. 1999; Hartnett and Wilson 1999, 2002; Clark et al. 2007). This study experimentally manipulated several factors known to impact plant community structure in native grasslands (N availability, soil depth and light availability) in order to assess their effects on restored plant communities. By manipulating resource availability (enhanced and reduced N availability and enhanced light availability) and soil depth (mimicking variation in soil depth with topography in native prairie), we attempted to determine what factors were affecting plant productivity and promoting or limiting diversity in this restored plant community.

Mowing effect

In native tallgrass prairie, light availability is an important resource promoting plant diversity and maintaining species richness (Collins et al. 1998), and mowing has been shown to be an effective method to increase light availability (Maron and Jefferies 2001; Tix and Charvat 2005). In our study, mowing increased light availability in both the altered nutrient availability and altered soil depth plots. In the altered nutrient availability plots, light interception by the regrowing canopy one month after mowing was greater in the enriched-N treatment, relative to ambient and reduced-N (Figure 3-3), while in the altered soil depth plots a significant interaction occurred where light availability was less in deep-unmowed plots compared to other soil depth-mowing combinations (Figure 3-2). Thus, mowing in combination with the other treatments created a range of light availability. Even though mowing altered light availability among the treatments, mowing actually decreased forb cover and productivity in the altered nutrient availability plots, and decreased forb cover in 2006 in the altered soil depth plots (Tables 3-5 and 3-6). In both the altered nutrient availability and soil depth plots, mowing did not lead to significant differences in richness, evenness, or diversity compared to unmowed plots. The negative effects of mowing on forb productivity and lack of response in plant community indices may be attributed to large quantities of dead biomass that remained on the plots after mowing which could have contributed to the overall low regrowth of forbs during the growing season. Furthermore, mowing occurred in June after many of the early season forbs flowered; therefore, these forbs did not respond positively to the mowing treatment (e.g. *Baptisia* spp.) resulting in less forb biomass compared to unmowed plots at the end of the growing season.

Nutrient and soil depth effects

Nitrogen is often limiting in tallgrass prairie (Risser and Parton 1982; Blair et al. 1998), and N additions have been shown to increase plant productivity (Seastedt et al. 1991; Baer et al. 2003), alter plant community structure (Gibson et al. 1993), and reduce species diversity through increased productivity of the dominant grasses (Wilson and Tilman 1991; Collins et al. 1998; Piper et al. 2005; Clark et al. 2007; Clark and Tilman 2008). In our study, the enriched-N treatment increased N availability which led to increased % grass cover and biomass, and increased total plant cover and total ANPP (Figure 3-7, Tables 3-2, 3-5, 3-6), while the reduced-N treatment tended to decrease N availability, though the effect was not statistically significant.

Baer and others (2003) found similar results in an earlier study in these plots, with grass biomass increasing in response to the enriched-N treatment.

Topographic variation alters plant-rooting depth, with shallow upland soils having greater N availability compared to deep lowland soils (Turner et al. 1997; Knapp et al. 1998). For N availability in our study, the altered soil depth treatments did not lead to significant differences between deep and shallow soils. This result was similar to earlier findings from these plots where no measured belowground resources differed between the soil depth treatments (Baer et al. 2003).

In native prairie, productivity is linked to resource availability. Productivity is highest in deep soil lowlands or areas enriched with nitrogen while shallow soil uplands or nutrient poor areas have decreased productivity (Gibson and Hulbert 1987; Collins 1992; Collins et al. 1998). From this observation we hypothesized that the enriched-unmowed treatment would have the greatest productivity in the altered nutrient availability plots, and the deep-unmowed treatment would have the greatest productivity in the altered soil depth plots compared to reduced-mowed treatment and shallow-mowed treatments, respectively. However, we found no significant treatment × mowing interaction in either the altered nutrient or altered soil depth plots.

Yearly differences occurred for aboveground net primary productivity (ANPP) due to differences in precipitation, with 2007 having the greatest ANPP across all treatments (585.91 ± 74.15 g m^{-2}). Within a given year, no differences in ANPP were observed among the altered nutrient availability treatments, while ANPP in deep soils was greater than in shallow soils in 2007 only. When comparing data on ANPP from this study to earlier results from the same restoration experiment (across all treatments = 660.0 ± 38.0 g m^{-2}; Baer et al. 2003), ANPP during this study was lower, except for 2007 enriched-N plots (846.21 ± 111.57 g m^{-2}; Table 3-5). When compared to the 22-year (1984-1996) average ANPP in nearby annually burned lowlands (527.5 ± 26.9 g m^{-2}) and annually burned uplands (369.1 ± 21.5 g m^{-2}) at KPBS (Knapp et al. 1998), which are also greatly influenced by dominant C_4 grasses (Towne and Owensby 1984), ANPP values from the altered nutrient availability and altered soil depth treatments fell within the range of ANPP values recorded (lowlands = 279 to 785 g m^{-2}, uplands = 178 to 570 g m^{-2}; Knapp et al. 1998) except for 2007 enriched-N plots (Table 3-5). These results suggest that the resource manipulations were adequate in altering ANPP to values comparable to native prairie with varying topographic positions (lowlands vs. uplands).

During the early stages of this restoration experiment, productivity was strongly affected by nitrogen addition while productivity in the reduced-N and ambient-N treatments was similar (Baer et al. 2003). This pattern in productivity among the altered nutrient availability treatments was also observed in the current study with regard to total ANPP across years and grass biomass. Baer and others (2003) concluded that similarity in ANPP of the reduced-N and ambient-N treatments was due to the high N use efficiency and low N requirements of the dominant grasses. In the altered soil depth plots, shallow soils resulted in lower grass productivity and lower total ANPP across all years (Table 3-5). This is similar to native prairie on shallow soil uplands which are less productive than deep soil lowlands (Knapp et al. 1993, 1998).

Patterns of forb productivity were not consistent with our expectations based on patterns observed in native prairie. We expected forb productivity to be greatest in the shallow soil or in the reduced-N treatments, and to benefit from the mowing treatment (Maron and Jefferies 2001). However, forb productivity was reduced with mowing in both the altered nutrient availability and altered soil depth plots, contrary to our prediction. In the altered soil depth treatment, no significant differences occurred between deep and shallow soil for forb productivity. Baer and others (2003) reported similar results with a lack of productivity responses to soil depth treatments. They attributed the lack of forb response to the inadequate competitive release from the dominant grasses, since the shallow soils had high ANPP (591 ± 57 g m^{-2}) in the early years of the experiment, even compared to the 22-yr average of 527.5 ± 26.9 for deep, productive lowlands at KPBS (Knapp et al. 1998; Baer et al. 2003). In this study, ANPP in the shallow soil treatment (average across years = 398.27 ± 59.96 g m^{-2}) was reduced substantially from the values reported by Baer et al. (2003), but remained greater than the 22-year average for shallow upland soils at KPBS (369.1 ± 21.5 g m^{-2}). The relatively high ANPP values in the shallow soil treatment may explain the lack of forb response to the shallow soil treatment.

Plant community response

In native prairie, greater N availability leads to increased productivity of the dominant grasses resulting in decreased species richness and diversity (Wilson and Tillman 2001; Collins et al. 1998; Piper et al. 2005; Clark et al. 2007; Clark and Tilman 2008). In our study, this relationship did not occur. The enriched-N treatment did lead to increased grass productivity; however, species diversity and richness were not statistically different from the non-enriched

treatments. In addition, the reduced-N treatment failed to significantly reduce grass productivity compared to ambient conditions, and did not lead to increased diversity. Although not statistically significant, species diversity was highest in the reduced-N treatment (H' = 1.49), which is consistent with findings from nutrient-poor native prairies. In contrast, early in this restoration experiment, Baer and others (2003) reported the enriched-N treatments increased productivity which led to decreased diversity, while C amendment with sawdust reduced productivity which increased species diversity. When comparing species richness values reported by Baer and others (2003), our values were lower in all nutrient treatments, which could correspond to several early-successional ruderal species being replaced over time by a few late-successional competitive species. For the altered soil depth plots, the shallow soil treatment had reduced productivity compared to deep soil and to the long-term average at KPBS, but this decrease in productivity did not result in increased plant species diversity, richness or evenness. Furthermore, species diversity values from the resource manipulation in this restoration experiment were still less than species diversity observed in native prairie on similar soil and topography (H'= 1.81).

Influence of Andropogon gerardii

In native prairie, the most productive sites typically have the greatest cover of the dominant C_4 grasses (Towne and Owensby 1984), but have decreased plant species diversity (Gibson and Hulbert 1987; Collins 1992; Collins et al. 1998). In this study, the lack of response in plant species diversity and richness with the manipulation of nutrient availability, soil depth, and mowing may have been influenced by the cover of the dominant grass, *A. gerardii* (Figure 3-9), which had average cover values up to 80% in some plots. Species diversity had a positive trend with cover of *A. gerardii*, contrary to our prediction and to other studies. McCain (this dissertation; Chapter 2) found that decreasing the cover *A. gerardii* by physical removal increased light availability allowing subordinate forb species to increase, leading to an increase in plant species diversity and richness. Other studies using different dominant species also suggest a general pattern whereby decreasing the cover or abundance of a dominant species led to increased diversity. An experiment by Gurevitch and Unnasch (1989) showed that removing *Dactylis glomerata* from an old field resulted in increased species diversity, evenness, and richness, as subordinate species increased in abundance and frequency. The positive relationship

between cover of *A. gerardii* and plant species diversity we observed (Figure 3-8) may be driven by interannual variability in climate. When each year was examined separately, the positive relationship was not significant or did not occur (2005: r = 0.6127, p = 0.1228; 2006: r = -0.3456, p = 0.6094; 2007: r = -0.4491, p = 0.5915).

Inouye and Tilman (1995) offer another explanation of why manipulating resources may fail to increase species richness and diversity. They found that if a dominant species is present at the onset of different resource manipulations, community convergence can occur because the dominant species prevents new subordinate species from establishing and the dominant species continues to increase in proportional abundance (Inouye and Tilman 1995). At the start of this restoration experiment, *A. gerardii* was abundant across all treatments and was the dominant species, with up to 80% cover in some areas. Even though we seeded additional forb species in 2005, these species did not establish well within the treatments. The dominance of *A. gerardii* across all treatments may have been the reason for poor forb establishment.

Conclusions

Native tallgrass prairie plant communities are usually dominated by a few grass species intermingled with a large number of varying subordinate species in low abundance (Gotelli and Simberloff 1987), which comprise the bulk of the overall species diversity of these communities (Collins and Glenn 1990). During restoration, excessive dominance of the grasses becomes a problem leading to overall low species richness and diversity. Manipulating the factors that decrease grass dominance may provide a competitive release for the subordinate species (Collins 1987; Hartnett et al. 1996; Howe 1994, 1999). In order to achieve and maintain the desired diversity in tallgrass prairie restorations, the dominance of the native grasses needs to be inhibited (Baer et al. 2005). Overall, species diversity and richness were not affected during the three years of this study by manipulations of soil nutrient availability, soil depth, or mowing, which may be related to the high abundance of *A. gerardii* across all treatments. This suggests that manipulation of soil resources (N availability or soil depth) may not be sufficient to inhibit the dominance of the C_4 grass, at least within the time frame of this study. Longer-term management may be required to detect significant increases in forb productivity and cover, or further management may be required in order to enhance plant species diversity and richness in this grass-dominated prairie restoration. Management such as an altered fire regime (e.g.

extended periods without prescribed fire) may be helpful in reducing the dominant grasses and promoting forb species (Howe 1994, 1995, 1999).

This experiment suggests that soil nutrient conditions in an established restoration may not be as important in affecting plant community dynamics, as compared to initial soil condition at the onset of a restoration. In the establishment phase of this experiment, community composition was greatly influenced by the effects of soil N availability on aboveground plant productivity, and in turn, light availability which affected diversity (Baer et al. 2003). However, several years later, the manipulation of the same soil resources plus addition of a mowing treatment did not significantly alter plant species diversity. Nitrogen availability and light availability continued to be important factors regulating productivity, but subordinate forb species failed to increase in response to resource manipulations. Inconsistency between the effects of resource manipulations on diversity in the early and late stages of a restoration may be attributed to inadequate competitive release of forbs in the presence of a dominant grass species, which is successful across a wide range of available resources. Thus, understanding how the subordinate forb species compete with the dominant species can be useful in guiding tallgrass prairie restorations.

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Figures and Tables

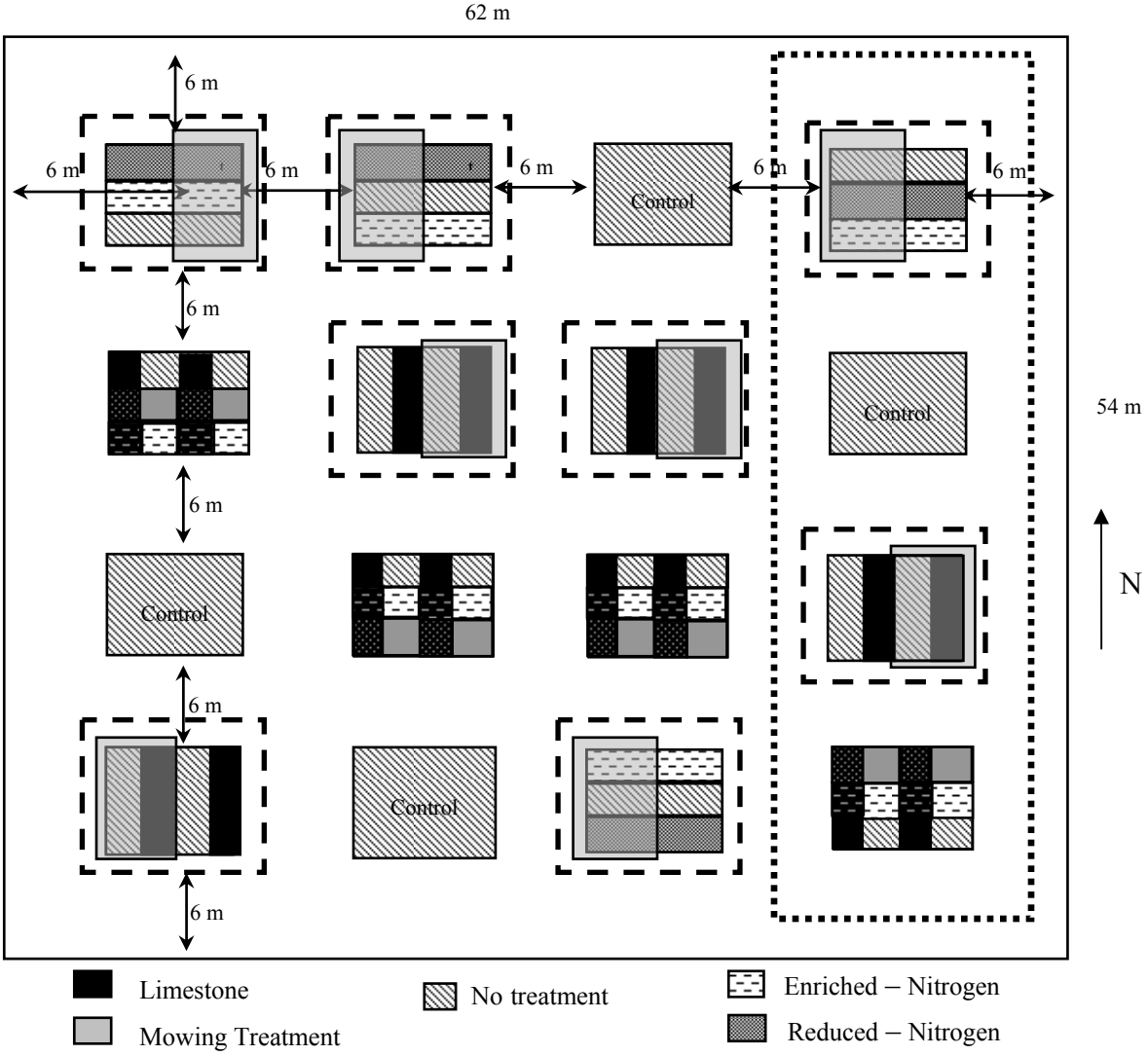

Figure 3-1. Layout of prairie restoration experiment established in 1998. Experimental design includes control plots, altered soil depth plots, altered nutrient availability plots, and plots with both altered soil depth and nutrient availability. Soil depth (deep and shallow) treatments and nutrient availability (reduced-, ambient-, and enriched-N) treatments were assigned to horizontal 2 × 6 m and vertical 2 × 8 strips, respectively. Each whole plot treatment was randomly assigned within each of four blocks (dotted outline) (n=4 per whole plot treatment). Only whole plot treatments outlined in a heavy dashed line (altered soil depth only or altered nutrient availability only) were used in this study. Each whole plot was divided into twelve 2 × 2 m subplots for sampling (n = 96 subplots total), and an annual mowing treatment was implemented in half of the plots starting in 2005.

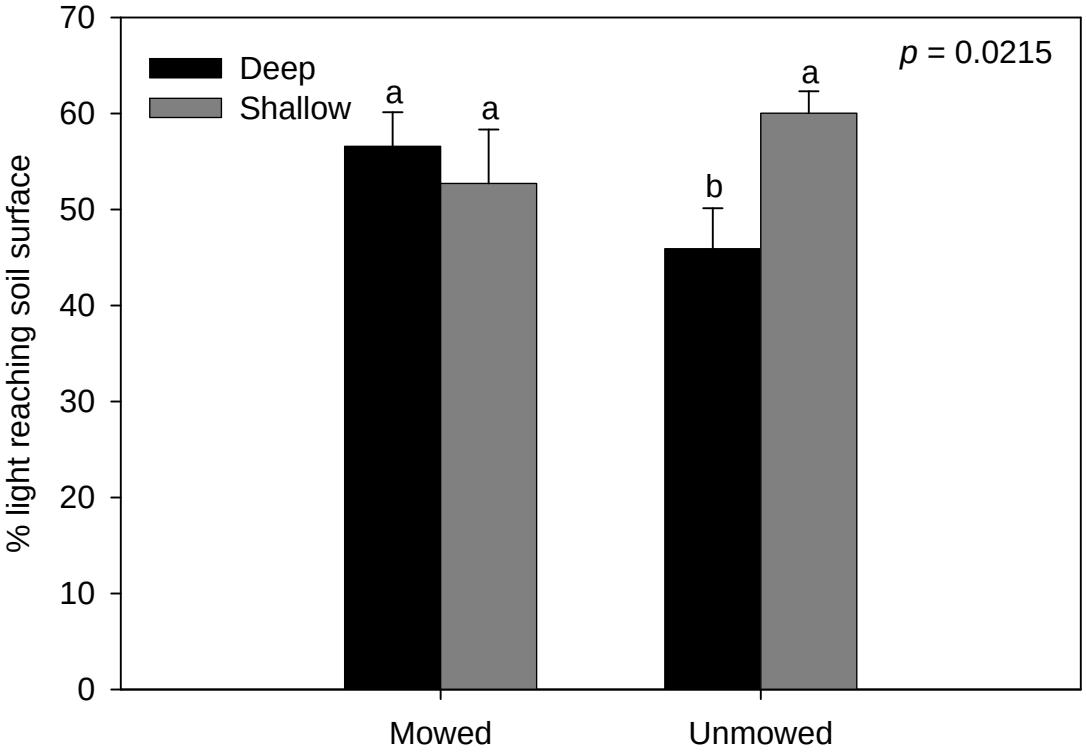

Figure 3-2. Means (± SE) of pre-mowing (June) light availability in 2007. There was significant mowing × soil depth interaction. Means accompanied by the same letter were not significantly different ($\alpha = 0.05$).

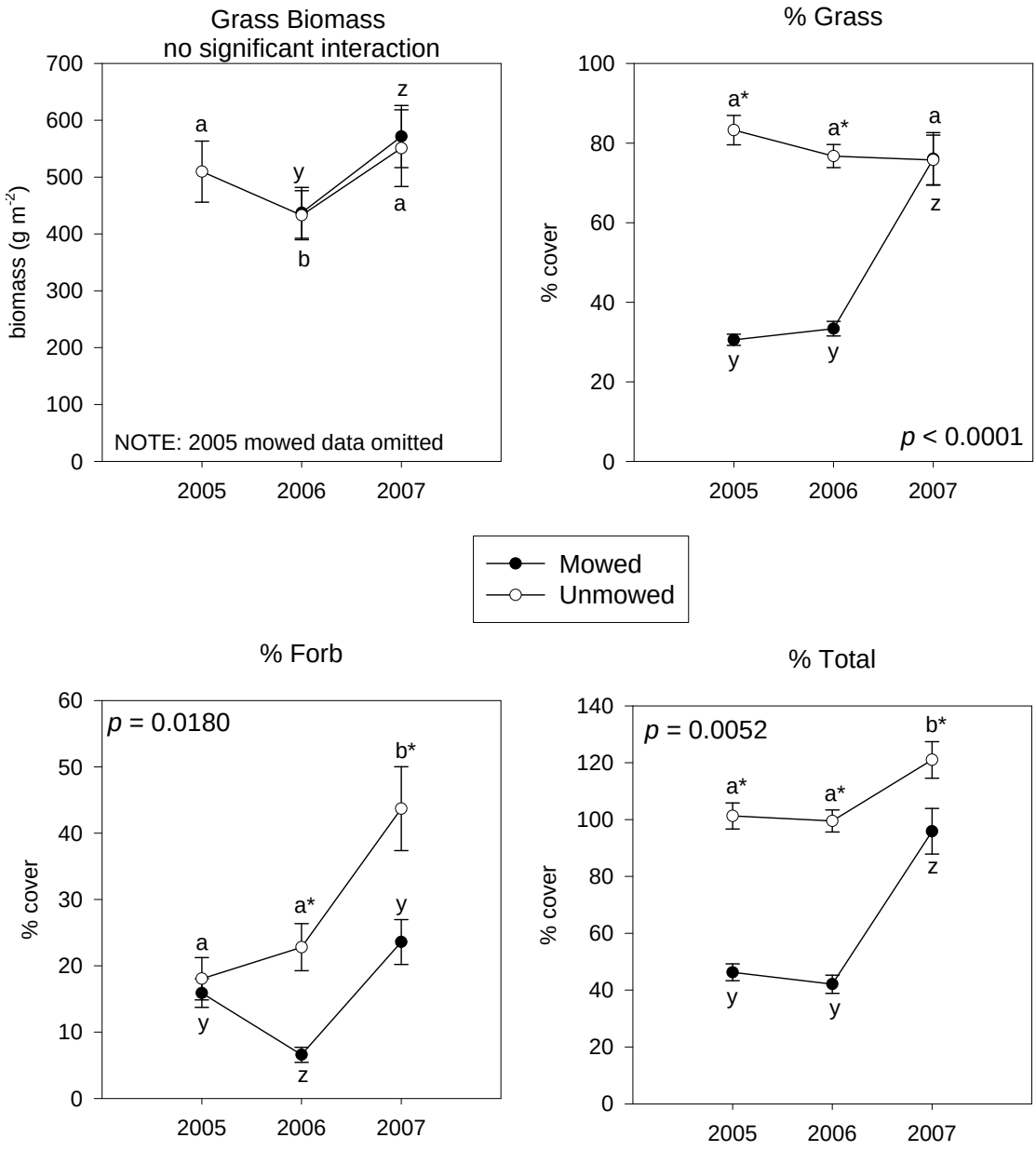

Figure 3-3. Means (± SE) of grass biomass, grass cover, forb cover and total cover for mowed and unmowed treatments in altered nutrient availability plots by year (2005-2007). *P*-values refer to significant mowing × year interactions. Significant differences in unmowed plots (open circles) are indicated by letters a-b and differences in mowed plots (closed circles) are indicated by letters x-z. Asterisks (*) depict significant differences between mowing treatments in a given year. Means accompanied by the same letter were not significantly different ($\alpha = 0.05$).

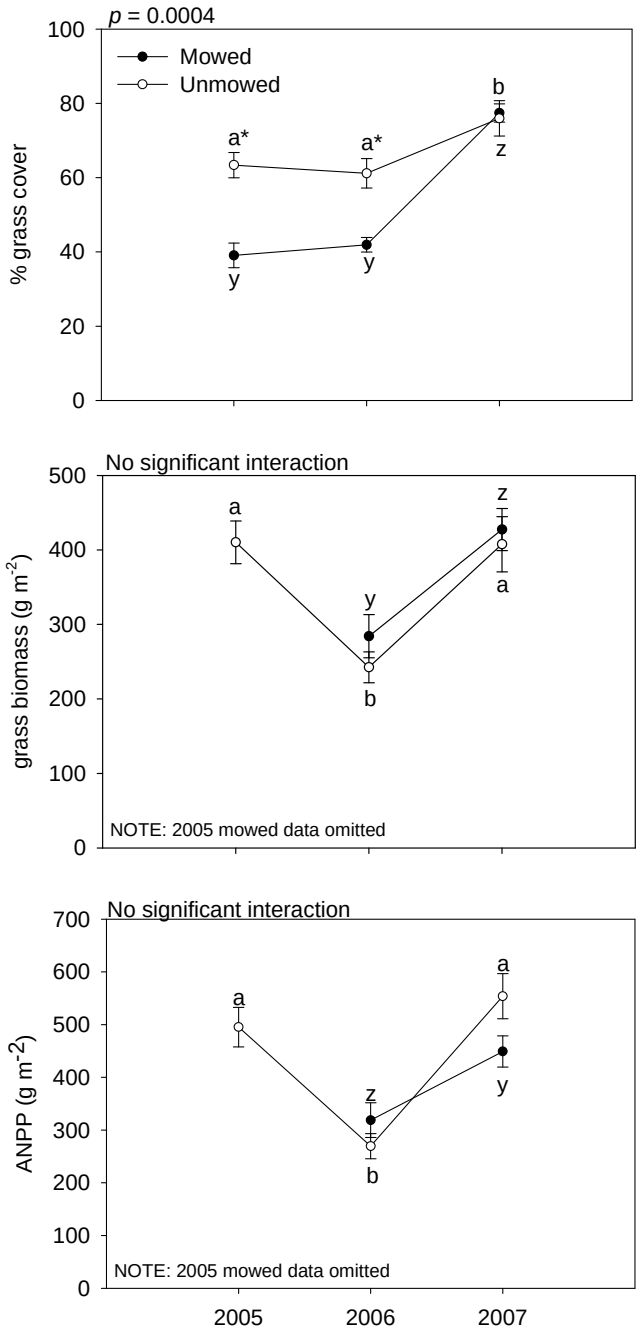

Figure 3-4. Means (± SE) of grass cover, grass biomass, and ANPP (excluding litter) for mowed and unmowed treatments averaged over deep and shallow soils over all years (2005-2007). *P*-values represent significant mowing × year interactions. Due to lack of pre-mowing biomass harvest, 2005 biomass data were omitted from grass biomass and ANPP resulting in the interaction being non-significant. Significant differences in unmowed plots (open circles) are indicated by letters a-b and differences in mowed plots (closed circles) are indicated by letters y-z. Asterisks (*) depict significant differences between mowing treatments in a given year. Means accompanied by the same letter were not significantly different ($\alpha = 0.05$).

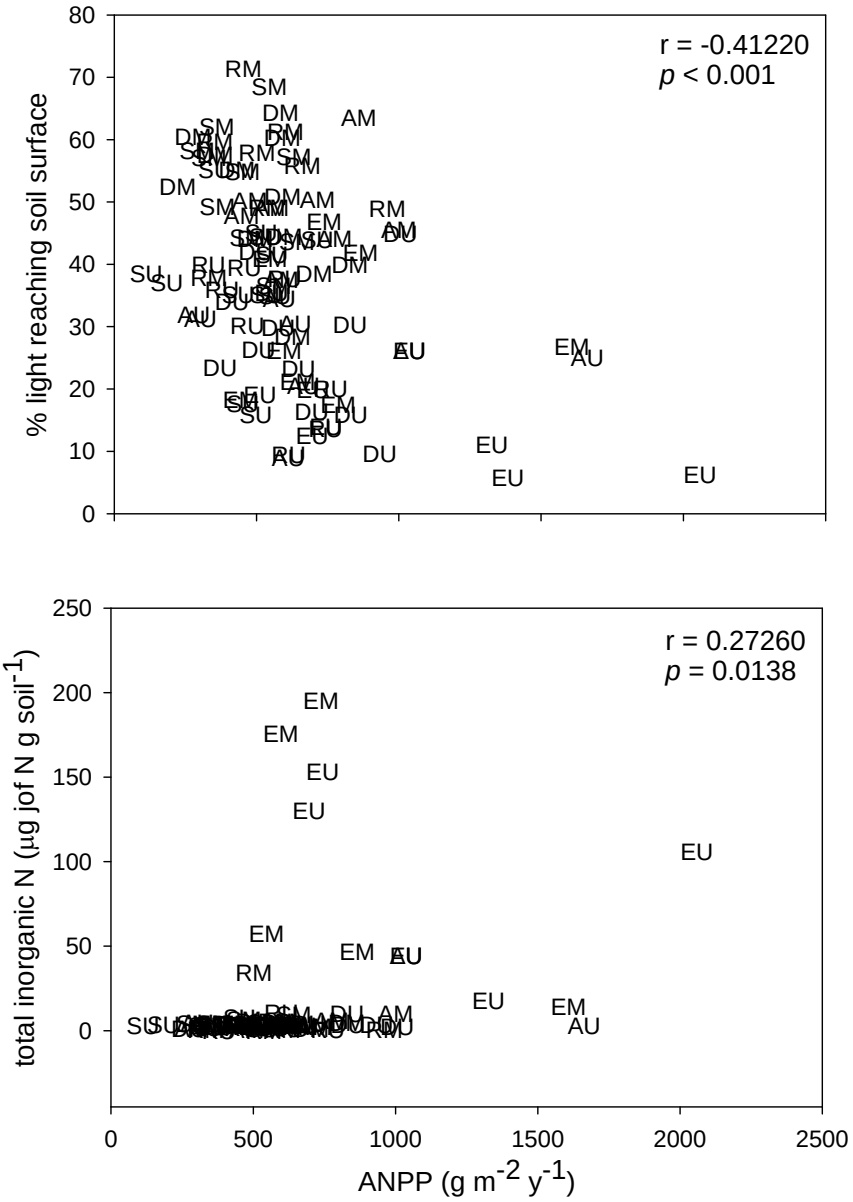

Figure 3-5. Correlations between ANPP and July light transmission (top panel), and ANPP and total inorganic N collected on ion exchange resins over the growing season (bottom panel) in 2007. Correlations were performed using data from individual subplots for both altered soil depth and altered nutrient availability treatments. Labels represent the subplot treatment (SM= shallow soil mowed; SU = shallow soil unmowed; DM = deep soil mowed; DU = deep soil unmowed; EM = enriched-N mowed; EU = enriched-N unmowed; AM = ambient-N mowed; AU = ambient-N unmowed; RM = reduced-N mowed; and RU = reduced-N unmowed). Significant relationships were determined from the Pearson correlation coefficient (r) derived using SAS (SAS 2002-2003).

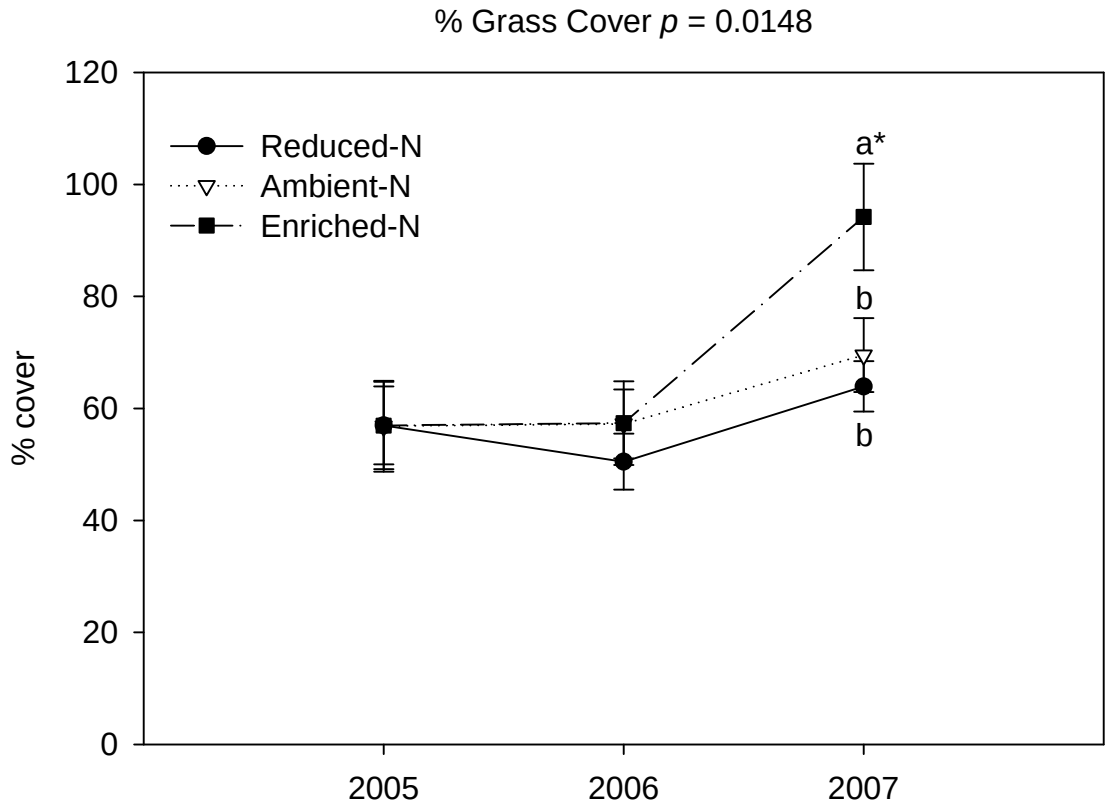

Figure 3-6. Means (± SE) of grass cover in altered nutrient availability treatments over all years (2005-2007). *P*-value is for a significant nutrient × year interaction term. Significant differences in reduced-N (closed circle), ambient-N (open triangle), and enriched-N treatments (closed square) occurred in 2007, but not in 2005 or 2006. Means accompanied by an asterisk (*) represent significant differences over all treatments between years, while means accompanied by differing letters represent significant differences between treatments within a given year (α = 0.05).

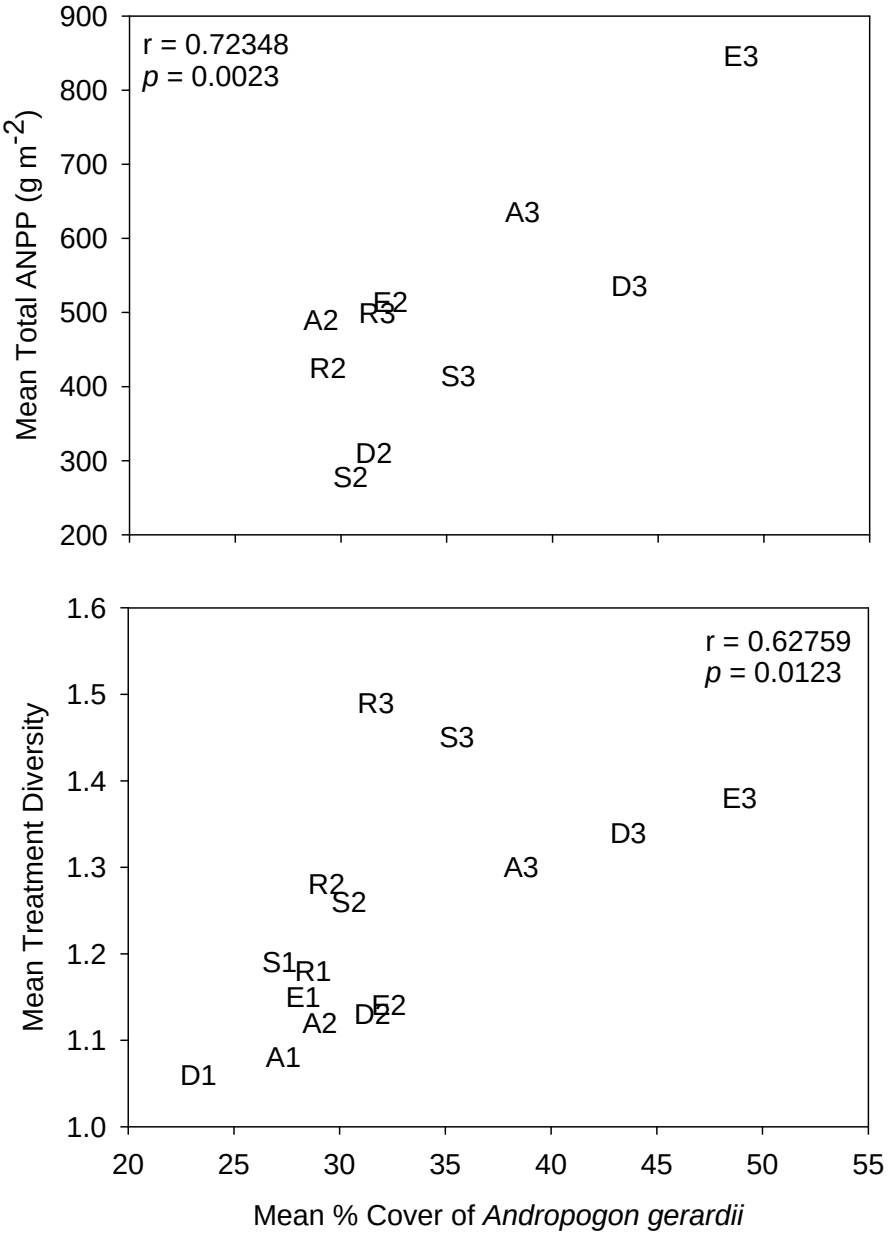

Figure 3-7. Correlations between mean % cover of *Andropogon gerardii* and mean total ANPP excluding litter (g m^{-2}) (top panel) and mean treatment diversity (Shannon diversity) (bottom panel) by year. All plots and years were included in the analyses, except for ANPP in 2005 due to lack of pre-mowing biomass sampling. Labels represent treatment (S = shallow soil, D = deep soil, R = reduced-N, A = ambient-N, and E = enriched-N) and year (1 = 2005, 2 = 2006, and 3 = 2007). Significant relationships were determined from the Pearson correlation coefficient (r) derived from SAS (SAS 2002-2003).

Table 3-1. Plant species added in 2005 at a seeding rate of 25 seeds m^{-2}. Nomenclature follows USDA, NRCS Plants Database (2007).

Species name	Species name
Achillea millefolium	*Oenothera biennis*
Asclepias tuberosa	*Penstemon tubiflorus*
Delphinium virescens	*Rudbeckia hirta*
Desmodium illinoense	*Silphium laciniatum*
Eupatorium altissimum	*Solidago speciosa*
Heliopsis helianthoides	*Symphyotrichum laeve*
Monarda fistulata	*Teucrium canadense*
	Tradescantia bracteata

Table 3-2. Means (± SE) of resin-collected inorganic N (μg N bag^{-1}) and mid-summer (July) soil water content by treatment within altered soil nutrient availability and altered soil depth plots. Data are presented by year. For the altered nutrient availability plots (A) there were significant nutrient main effects of treatments on total inorganic N, NH_4-N, NO_3-N and soil water content, but mowing did not affect soil N availability (n=6 per treatment combination per year). For the altered soil depth plots (B) there were significant main effects of both soil depth and mowing treatments on soil water content (n=4 per treatment combination per year), but not on soil N availability. There were no significant interactions (nutrient × mow or soil depth × mow). Means within a year with the same letter were not significantly different (α = 0.05).

		Year 1 (2005)	Year 2 (2006)	Year 3 (2007)
A) Altered Nutrient Availability Plots				
Total Inorganic N	Reduced-N	499 (85)[a]	…	384 (268)[a]
	Ambient-N	1702 (964)[a]	…	645 (331)[a]
	Enriched-N	7356 (1861)[b]	…	15581 (4953)[b]
	Type III F, *p*	0.0024	…	0.0210
NH_4-N	Reduced-N	415 (70)[a]	…	299 (236)
	Ambient-N	498 (103)[a]	…	192 (84)
	Enriched-N	845 (172)[b]	…	948 (355)
	Type III F, *p*	0.0295	…	0.2713
NO_3-N	Reduced-N	84 (25)[a]	…	85 (44)[a]
	Ambient-N	1204 (926)[a]	…	412 (265)[b]
	Enriched-N	6510 (1769)[b]	…	14008 (4948)[c]
	Type III F, *p*	0.0025	…	0.0097
Soil Water Content (%)	Reduced-N	24.80 (0.94)	19.34 (0.90)[a]	18.90 (0.84)
	Ambient-N	23.83 (0.63)	17.69 (1.06)[ab]	17.07 (0.67)
	Enriched-N	24.45 (0.63)	15.53 (0.75)[b]	16.77 (0.61)
	Type III F, *p*	0.3287	0.0200	0.0860

	Mowed	24.30 (0.59)	18.55 (0.91)[a]	18.21 (0.56)
	Unmowed	24.31 (0.59)	16.50 (0.90)[b]	16.83 (0.55)
	Type III F, *p*	0.9959	0.0396	0.1476
B) Altered Soil Depth Plots				
Total Inorganic N	Shallow soil	12.06 (2.87)	…	3.57 (0.55)
	Deep soil	8.59 (1.04)	…	3.32 (0.48)
	Type III F, *p*	0.1184	…	0.7002
NH_4-N	Shallow soil	6.06 (1.12)	…	1.46 (0.47)
	Deep soil	5.05 (0.45)	…	1.47 (0.43)
	Type III F, *p*	0.2791	…	0.9761
NO_3-N	Shallow soil	5.99 (2.10)	…	2.11 (0.24)
	Deep soil	3.54 (0.72)	…	1.85 (0.24)
	Type III F, *p*	0.1270	…	0.3146
Soil Water Content (%)	Shallow soil	23.61 (0.40)	15.66 (0.51)[a]	10.48 (0.30)[a]
	Deep soil	23.20 (0.31)	16.87 (0.51)[b]	11.46 (0.28)[b]
	Type III F, *p*	0.0894	0.0037	0.0022

Table 3-3. Mean (± SE) concentrations of inorganic soil N (μg N g soil^{-1}) from soil cores (0-10 cm) collected in July by treatment within altered soil nutrient availability and altered soil depth plots. Data are presented by year as well as averaged across years (nutrient, soil depth, or mowing main effects) and averaged over all treatments within a given year (year main effect). For the altered nutrient availability plots (A), the nutrient main effect was significant, but the mowing main effect was not (n=6 per treatment combination per year). For the altered soil depth plots (B), there were significant main effects of both soil depth and mowing treatments (n=4 per treatment combination per year). Within each year, significant differences among treatments are indicated by different numbers. Significant main effects occurred for year or treatment (nutrient, soil depth, or mowing); differences among treatments (over all years) are indicated by letters a-c and differences among years (over all treatments) are indicated by letters x-z. There were no significant interactions. Means accompanied by the same letter were not significantly different ($\alpha = 0.05$).

	Year 1 (2005)	Year 2 (2006)	Year 3 (2007)	Across all years
A) Altered Nutrient Availability Plots				
Total inorganic N				($p = 0.0074$)
Reduced-N	0.90 (0.09)[1]	3.52 (0.78)[1]	1.86 (0.36)	2.09 (0.76)[a]
Ambient-N	1.55 (0.35)[1]	4.46 (0.63)[1]	3.34 (0.81)	3.12 (0.85)[a]
Enriched-N	3.74 (1.21)[2]	6.09(0.56)[2]	4.01 (0.56)	4.61 (0.74)[b]
Type III F, *p*	0.0506	0.0290	0.0974	
Over all treatments ($p < 0.0001$)	2.06 (0.86)[x]	4.69 (0.75)[y]	3.07 (0.63)[z]	
NH_4-N				($p = 0.1665$)
Reduced-N	0.53 (0.08)	2.31 (0.74)	1.05 (0.37)	1.30 (0.53)
Ambient-N	1.00 (0.28)	2.45 (0.55)	1.45 (0.48)	1.63 (0.43)
Enriched-N	1.36 (0.49)	3.02 (0.45)	2.06 (0.97)	2.15 (0.48)
Type III F, *p*	0.2008	0.05236	0.5047	
Over all treatments ($p <0.0001$)	0.96 (0.24)[y]	2.59 (0.22)[z]	1.52 (0.29)[y]	

NO_3-N				(p = 0.0035)
Reduced-N	0.37 (0.03)[1]	1.21 (0.07)[1]	0.81 (0.01)[1]	0.80 (0.24)[a]
Ambient-N	0.55 (0.11)[1]	2.02 (0.23)[1]	1.87 (0.39)[1,2]	1.48 (0.47)[b]
Enriched-N	2.38 (0.77)[2]	3.07 (0.43)[2]	4.00 (1.35)[2]	3.15 (0.47)[c]
Type III F, p	0.0275	0.0064	0.0485	
Over all treatments (p = 0.0033)	1.10 (0.64)[y]	2.10 (0.54)[z]	2.23 (0.94)[z]	

B) Altered Soil Depth Plots

Total inorganic N				(p = 0.8240)
Shallow soil	1.38 (0.49)	4.00 (0.34)	3.29 (0.24)	2.78 (0.71)
Deep soil	1.27 (0.23)	3.68 (0.40)	3.27 (0.25)	2.85 (0.81)
Type III F, p	0.7759	0.3526	0.8148	
Over all treatments (p < 0.0001)	1.33 (0.05)[x]	3.84 (0.16)[y]	3.27 (0.01)[z]	

				(p = 0.0340)
Mowed	1.42 (0.30)	4.36 (0.46)	3.68 (0.27)	3.15 (0.89)[a]
Unmowed	1.23 (0.42)	3.32 (0.28)	2.87 (0.22)	2.47 (0.63)[b]
Type III F, p	0.6439	0.0581	0.0876	
Over all treatments (p < 0.0001)	1.33 (0.09)[x]	3.84 (0.52)[y]	3.27 (0.41)[z]	

NH_4-N				(p = 0.6436)
Shallow soil	0.63 (0.28)	2.06 (0.33)	1.73 (0.19)	1.47 (0.43)
Deep soil	0.65 (0.18)	2.10 (0.33)	1.42 (0.13)	1.39 (0.42)
Type III F, p	0.9294	0.8866	0.0516	
Over all treatments (p < 0.0001)	0.64 (0.01)[x]	2.08 (0.02)[y]	1.58 (0.16)[z]	

NO_3-N				(p = 0.2698)
Shallow soil	0.62 (0.14)	1.94 (0.13)	1.56 (0.20)[1]	1.41 (0.35)
Deep soil	0.75 (0.23)	1.90 (0.12)	1.85 (0.23)[2]	1.45 (0.42)
Type III F, p	0.4879	0.3260	0.0208	
Over all treatments	0.68 (0.06)[y]	1.92 (0.02)[z]	1.70 (0.15)[z]	
(p < 0.0001)	----------			
				(p = 0.0021)
Mowed	0.87 (0.28)	2.22 (0.17)[1]	1.81 (0.21)	1.64 (0.39)[a]
Unmowed	0.49 (0.08)	1.62 (0.08)[2]	1.59 (0.22)	1.24 (0.37)[b]
Type III F, p	0.1325	0.0291	0.2642	
Over all treatments	0.68 (0.20)[y]	1.92 (0.30)[z]	1.70 (0.11)[z]	
(p < 0.0001)				

Table 3-4. Mean (± SE) concentrations of inorganic soil N (μg N g soil^{-1}) from soil cores (0-10 cm) collected in September by treatment within altered soil nutrient availability and altered soil depth plots. Data are presented by year as well as averaged across years (nutrient, soil depth, or mowing main effects) and averaged over all treatments within a given year (year main effect). For the altered nutrient availability plots (A), the nutrient main effect was significant, but the mowing main effect was not (n=6 per treatment combination per year). For the altered soil depth plots (B), the soil depth main effect was significant, but the mowing main effect was not (n=4 per treatment combination per year). Within each year, significant differences among treatments are indicated by different numbers. Significant main effects occurred for year or treatment (nutrient or soil depth); differences among treatments (over all years) are indicated by letters a-b and differences among years (over all treatments) are indicated by letters x-z. There were no significant interactions. Means accompanied by the same letter were not significantly different (α = 0.05).

	Year 1 (2005)	Year 2 (2006)	Year 3 (2007)	Across all years
A) Altered Nutrient Availability Plots				
Total inorganic N				(p = 0.0336)
Reduced-N	1.66 (0.33)	3.51 (0.42)[1]	3.21 (0.40)	2.79 (0.57)[a]
Ambient-N	2.96 (0.55)	5.30 (0.66)[2]	5.48 (1.01)	4.58 (0.81)[b]
Enriched-N	3.20 (0.29)	5.56 (0.58)[2]	6.22 (0.90)	5.00 (0.92)[b]
Type III F, p	0.0551	0.0359	0.3010	
Over all treatments (p < 0.0001)	2.61 (0.48)[y]	4.79 (0.65)[z]	4.97 (0.91)[z]	
NH_4-N				(p = 0.1904)
Reduced-N	1.66 (0.34)	2.75 (0.38)	2.97 (0.40)	2.46 (0.41)
Ambient-N	2.18 (0.41)	3.94 (0.61)	3.48 (0.61)	3.20 (0.53)
Enriched-N	2.40 (0.29)	3.54 (0.35)	2.55 (0.23)	2.83 (0.35)
Type III F, p	0.2191	0.1259	0.2553	
Over all treatments (p <0.0001)	2.08 (0.22)[x]	3.41 (0.35)[y]	3.00 (0.27)[z]	

NO_3-N				(p = 0.0087)
Reduced-N	0.00 (0.01)[1]	0.76 (0.07)[1]	0.24 (0.01)	0.33 (0.22)[a]
Ambient-N	0.77 (0.29)[2]	1.35 (0.11)[1]	2.00 (0.49)	1.38 (0.35)[b]
Enriched-N	0.80 (0.18)[2]	2.02 (0.32)[2]	3.67 (0.96)	2.17 (0.83)[b]
Type III F, p	0.0221	0.0097	0.3332	
Over all treatments (p = 0.0033)	0.52 (0.26)[x]	1.38 (0.37)[y]	1.97 (0.99)[z]	

B) Altered Soil Depth Plots

Total inorganic N				(p = 0.2801)
Shallow soil	2.56 (0.44)	4.71 (0.35)[1]	4.32 (0.89)	3.86 (0.66)
Deep soil	3.61 (0.80)	6.20 (0.84)[2]	3.59 (0.33)	4.46 (0.87)
Type III F, p	0.1844	0.0145	0.3147	
Over all treatments (p < 0.0001)	3.08 (0.52)[y]	5.45 (0.74)[z]	3.96 (0.37)[y]	
NH_4-N				(p = 0.4393)
Shallow soil	1.98 (0.34)	3.46 (0.31)[1]	3.50 (0.75)	2.98 (0.50)
Deep soil	2.80 (0.71)	4.68 (0.68)[2]	2.65 (0.18)	3.38 (0.65)
Type III F, p	0.2329	0.0102	0.3147	
Over all treatments (p = 0.0011)	2.39 (0.41)[y]	4.07 (0.61)[z]	3.08 (0.42)[y]	
NO_3-N				(p = 0.0182)
Shallow soil	0.58 (0.16)	1.25 (0.10)	0.82 (0.23)	0.88 (0.20)[a]
Deep soil	0.81 (0.18)	1.52 (0.19)	0.94 (0.29)	1.09 (0.22)[b]
Type III F, p	0.1582	0.1090	0.3147	
Over all treatments (p < 0.0001)	0.70 (0.11)[y]	1.38 (0.13)[z]	0.88 (0.06)[y]	

Table 3-5. Means (± SE) of plant biomass (g m^{-2}) by treatment within altered soil nutrient availability and altered soil depth plots. Data are presented for each year as well as averaged across years (nutrient, soil depth, or mowing main effects) and averaged over all treatments within a given year (year main effect). For the altered nutrient availability plots (A) there were significant main effects of both nutrient and mowing treatments (n=6 per treatment combination per year). For the altered soil depth plots (B) there were significant main effects of both soil depth and mowing treatments (n=4 per treatment combination per year). Within each year, significant differences among treatments are indicated by different numbers. Significant main effects occurred for year or treatment (nutrient, soil depth, or mowing); differences among treatments (over all years) are indicated by letters a-b and differences among years (over all treatments) are indicated by letters x-z. Means accompanied by the same letter were not significantly different ($\alpha = 0.05$). NOTE: Total ANPP in 2005 included litter material, but excluded pre-mowing biomass samples. Total ANPP in 2006 and 2007 excluded litter material, but included pre-mowing biomass samples (see Methods for further details).

	Year 1 (2005)	Year 2 (2006)	Year 3 (2007)	Across all years
A) Altered Nutrient Availability Plots				
Grass Biomass (g m^{-2})				($p = 0.0032$)
Reduced-N	358.04[1] (42.61)	376.36 (36.46)	459.54[1] (38.83)	397.98[a] (31.23)
Ambient-N	348.76[1] (54.92)	446.88 (64.66)	501.69[1] (44.90)	432.44[a] (77.27)
Enriched-N	499.66[2] (63.56)	482.31 (54.44)	722.31[2] (105.84)	568.10[b] (77.27)
Over all treatments ($p = 0.0005$)	402.15[y] (48.83)	435.19[y] (31.14)	561.18[z] (81.48)	
Forb Biomass (g m^{-2})				($p = 0.0440$)
Mowed	6.21 (2.73)	27.93 (9.23)	34.3 (12.22)[1]	22.81 (8.5)[a]
Unmowed	14.68 (5.45)	54.37 (17.67)	163.47 (65.65)[2]	77.5 (44.5)[b]
Over all treatments ($p = 0.0201$)	10.44 (4.24)[y]	41.15 (13.2)[yz]	98.88 (64.58)[z]	

Total ANPP (g m^{-2})				(p = 0.0079)
Reduced-N	461.56 (43.44)	425.39 (39.15)	498.48 (39.54)	461.81[a] (21.10)
Ambient-N	466.21 (59.19)	489.52 (61.42)	635.51 (80.30)	530.41[a] (52.98)
Enriched-N	608.77 (58.49)	514.09 (56.00)	846.21 (111.57)	656.36[b] (98.78)
Over all treatments (p = 0.0005)	512.18[y] (48.31)	476.33[y] (26.44)	660.07[z] (101.13)	

B) Altered Soil Depth Plots

Grass Biomass (g m^{-2})				(p = 0.1190)
Deep Soil	327.86 (32.54)	279.92 (29.80)	453.19 (34.56)	353.65 (51.66)
Shallow Soil	317.23 (30.82)	246.74 (20.12)	377.64 (29.39)	313.87 (37.83)
Over all treatments (p < 0.0001)	322.55[x] (31.68)	263.33[y] (24.96)	415.41[z] (31.97)	
Forb Biomass (g m^{-2})				(p = 0.3568)
Mowed	23.62 (9.83)	34.57 (14.27)	21.70 (7.22)	26.63 (4.00)
Unmowed	36.62 (10.63)	27.00 (13.64)	95.59 (37.98)	53.07 (21.44)
Over all treatments (p = 0.1644)	30.12 (6.50)	30.78 (3.79)	58.64 (36.95)	
Total ANPP (g m^{-2})				(p = 0.1169)
Deep Soil	425.52 (34.33)	309.87 (35.23)	535.36[1] (36.99)	441.92 (81.39)
Shallow Soil	457.43 (35.94)	278.35 (20.92)	413.97[2] (27.52)	398.27 (59.96)
Over all treatments (p < 0.0001)	441.48[z] (35.14)	294.11[y] (28.07)	474.67[z] (32.25)	

Table 3-6. Means (± SE) of percent cover by treatment within altered soil nutrient availability and altered soil depth plots. Data are presented for each year as well as averaged across years (nutrient, soil depth, or mowing main effects) and averaged over all treatments within a given year (year main effect). For the altered nutrient availability plots (A) there were significant main effects of both nutrient and mowing treatments (n=6 per treatment combination per year). For the altered soil depth plots (B) only the mowing main effect was significant (n=4 per treatment combination per year). Within each year, significant differences among treatments are indicated by different numbers. Significant main effects occurred for year or treatment (nutrient or mowing); differences among treatments (over all years) are indicated by letters a-b and differences among years (over all treatments) are indicated by letters y-z. Means accompanied by the same letter were not significantly different ($\alpha = 0.05$).

	Year 1 (2005)	Year 2 (2006)	Year 3 (2007)	Across all years
A) Altered Nutrient Availability Plots				
% Cover of Grasses				($p = 0.0191$)
Reduced-N	56.97 (6.95)	50.50 (4.99)	63.94 (4.51)^{1}	57.14 (3.88)^{a}
Ambient-N	56.81 (8.11)	57.22 (6.17)	69.53 (6.59)^{1}	61.19 (4.17)^{a}
Enriched-N	56.93 (7.80)	57.38 (7.49)	94.19 (9.53)^{2}	69.50 (12.34)^{b}
Over all treatments	56.91 (0.05)^{y}	55.03 (2.27)^{y}	75.88 (9.29)^{z}	
($p < 0.0001$)	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -
				($p < 0.0001$)
Mowed	30.56 (1.42)^{1}	33.35 (1.83)^{1}	76.02 (6.60)	46.65 (14.71)^{a}
Unmowed	83.25 (3.71)^{2}	76.71 (2.90)^{2}	75.75 (6.22)	78.57 (2.36)^{b}
Over all treatments	56.91 (26.34)^{y}	55.03 (21.68)^{y}	75.88 (0.14)^{z}	
($p < 0.0001$)				
% Cover of Forbs				($p = 0.0784$)
Mowed	15.90 (2.16)	6.58 (1.14)^{1}	23.58 (3.39)^{1}	15.35 (4.91)
Unmowed	18.04 (3.19)	22.79 (3.55)^{2}	43.69 (6.32)^{2}	28.17 (7.88)
Over all treatments	16.97 (1.07)^{y}	14.69 (8.10)^{y}	33.64 (10.05)^{z}	
($p < 0.0001$)				

% Cover of Total				(p = 0.0005)
Mowed	46.29 (2.95)[1]	42.10 (3.19)[1]	95.90 (8.01)[1]	61.43 (17.27)[a]
Unmowed	101.2 (4.56)[2]	99.5 (3.87)[2]	121.02 (6.41)[2]	107.27 (6.89)[b]
Over all treatments	73.79 (27.5)[y]	70.80 (28.7)[y]	108.46 (12.56)[z]	
(p < 0.0001)				

B) Altered Soil Depth Plots

% Cover of Grasses				(p = 0.0238)
Mowed	39.06 (3.32)[1]	41.92 (1.95)[1]	77.41 (2.44)	52.80 (12.33)[a]
Unmowed	63.08 (3.41)[2]	61.17 (3.95)[2]	75.96 (4.77)	66.83 (7.98)[b]
Over all treatments	51.22 (12.16)[y]	51.54 (9.63)[y]	76.68 (0.72)[z]	
(p < 0.0001)				
% Cover of Forbs				(p = 0.2397)
Mowed	18.31 (2.39)	11.25 (3.02)[1]	24.68 (3.94)	18.08 (3.88)
Unmowed	20.75 (3.62)	19.79 (2.82)[2]	39.35 (4.93)	26.63 (6.37)
Over all treatments	19.53 (1.22)[y]	15.52 (4.27)[y]	32.02 (7.34)[z]	
(p < 0.0001)				
% Cover of Total				(p = 0.0003)
Mowed	57.38 (3.45)[1]	53.17 (3.85)[1]	102.09 (4.37)	70.88 (15.65)[a]
Unmowed	84.13 (4.42)[2]	85.96 (3.11)[2]	120.92 (5.18)	97.00 (11.97)[b]
Over all treatments	70.75 (13.38)[y]	69.56 (16.40)[y]	111.50 (9.41)[z]	
(p < 0.0001)				

Table 3-7. Means (± SE) of species richness (no. species 0.25 m^{-2}), evenness (J) and diversity (H') by treatment within altered soil nutrient availability and altered soil depth plots. Data are presented for each year, as well as averaged over all treatments within a given year (year main effect). For the altered nutrient availability plots n=6 per treatment combination per year, and for the altered soil depth plots n=4 per treatment combination per year. Within each year, significant differences among treatments are indicated by different numbers. Significant main effects occurred for year with differences among years (over all treatments) are indicated by letters x-z. Means accompanied by the same letter were not significantly different ($\alpha = 0.05$).

	Year 1 (2005)	Year 2 (2006)	Year 3 (2007)
A) Altered Nutrient Availability Plots			
Species Richness			
Reduced-N	5.13 (0.29)	4.89 (0.26)	5.75 (0.40)
Ambient-N	4.31 (0.33)	4.19 (0.23)	4.69 (0.25)
Enriched-N	4.63 (0.29)	4.00 (0.42)	5.63 (0.46)
Over all treatments ($p < 0.0001$)	4.69 (0.24)^{y}	4.35 (0.27)^{y}	5.35 (0.34)^{z}
Native prairie	…	…	8.00 (0.93)
Species Evenness			
Reduced-N	0.73 (0.02)	0.82 (0.02)	0.87 (0.02)^{1}
Ambient-N	0.77 (0.02)	0.79 (0.03)	0.86 (0.02)^{1}
Enriched-N	0.76 (0.03)	0.82 (0.02)	0.79 (0.01)^{2}
Over all treatments ($p < 0.0001$)	0.75 (0.01)^{y}	0.81 (0.01)^{z}	0.84 (0.03)^{z}
Native prairie	…	…	0.89 (0.02)

Species Diversity			
Reduced-N	1.18 (0.07)	1.28 (0.06)	1.49 (0.07)
Ambient-N	1.08 (0.06)	1.12 (0.06)	1.30 (0.05)
Enriched-N	1.15 (0.07)	1.14 (0.06)	1.38 (0.04)
Over all treatments ($p < 0.0001$)	1.14 (0.03)[y]	1.18 (0.05)[y]	1.39 (0.06)
Native prairie	…	…	1.81 (0.11)
B) Altered Soil Depth Plots			
Species Richness			
Deep soil	5.00 (0.35)	4.42 (0.28)	5.21 (0.34)
Shallow soil	5.20 (0.26)	5.00 (0.28)	5.45 (0.22)
Over all treatments ($p = 0.0621$)	5.10 (0.30)	4.71 (0.28)	5.33 (0.28)
Native prairie	…	…	8.00 (0.93)
Species Evenness			
Deep soil	0.71 (0.03)	0.75 (0.03)	0.84 (0.02)
Shallow soil	0.74 (0.03)	0.80 (0.02)	0.87 (0.02)
Over all treatments ($p < 0.0001$)	0.73 (0.03)[x]	0.78 (0.02)[y]	0.85 (0.02)[z]
Native prairie	…	…	0.89 (0.02)
Species Diversity			
Deep soil	1.06 (0.06)	1.13 (0.04)[1]	1.34 (0.05)
Shallow soil	1.19 (0.05)	1.26 (0.05)[2]	1.45 (0.04)
Over all treatments ($p < 0.0001$)	1.14 (0.05)[y]	1.19 (0.05)[y]	1.39 (0.04)[z]

Appendix 1. Original species and seeding rates used in a prairie restoration initiated in 1998. Nomenclature follows USDA, NRCS Plants Database (2007).

Dominant grasses (160 seeds m^{-2})	Common species (16 seeds m^{-2})
Andropogon gerardii	*Artemisia ludoviciana*
Panicum virgatum	*Bouteloua curtipendula*
Schizachyrium scoparium	*Salvia azurea*
Sorghastrum nutans	*Solidago canadensis*
	Symphyotrichum ericoides

Frequent species (10 seeds m^{-2})	Uncommon species (5 seeds m^{-2})
Amorpha canescens	*Asclepias viridis*
Asclepias verticillata	*Baptisia australis*
Brickellia eupatorioides	*Baptisia bracteata*
Ceanothus herbaceus	*Callirhoe involucrata*
Dalea purpurea	*Dalea candida*
Koeleria macrantha	*Desmanthus illoenisis*
Lespedeza capitata	*Echinacea angustifolia*
Mimosa nuttallii	*Liastris punctata*
Solidago missouriensis	*Lomatium foeniculaceum*
Sporobolus compositus	*Oenothera macrocarpa*
Sporobolus heterolepis	*Packera plattensis*
Symphyotrichum oblongifolium	*Penstemon cobaea*
Vernonia fasciculata	*Penstemon grandiflorus*
	Psoralidium tenuiflorum
	Ratibida columnifera
	Rosa arkansana
	Ruellia humilis
	Symphyotrichum sericeum
	Sisyrinchium campestre

CHAPTER 4 - INFLUENCE OF MYCORRHIZAE ON PLANT PRODUCTIVITY AND FORB ESTABLISHMENT IN A GRASS-DOMINATED PRAIRIE RESTORATION

Abstract

During the initiation of a tallgrass prairie restoration the ratio of grass to forb species in the seed mixture is typically chosen based on a desired plant community composition and species diversity. However, the warm-season C_4 grasses often become more dominant than desired, while establishment and survival of subordinate grass and forb species is less successful, leading to overall low richness and diversity. The dominant grasses in tallgrass prairie are strongly mycotrophic, while many subordinate species appear to be less dependent on mycorrhizal symbiosis. Therefore, manipulating arbuscular mycorrhizal (AM) fungi during tallgrass prairie restoration may be useful in promoting establishment and growth of forb species in grass-dominated tallgrass prairie restorations. A 2-year field experiment was conducted to assess the role of AM fungi in affecting plant species composition, productivity, cover, leaf tissue quality and diversity in restored tallgrass prairie, and to assess the effects of manipulation of mycorrhizae on sown forb species that vary in degree of mycorrhizal dependence. Plant productivity and community composition were quantified in replicate plots where either mycorrhizal fungi were suppressed by applying a fungicide (Fungo®; thiophanate-methyl) every two weeks during each growing season, or were subjected to a one-time application of a commercial AM fungal inoculum (Myco-bio-boost^{TM}). These treatment plots were compared to data from non-treated control plots in a prairie restoration established 8 years prior to the start of this study at Konza Prairie Biological Station. Since the effectiveness of Myco-bio-boost^{TM} commercial inoculum was unknown, a separate greenhouse study was conducted to compare root colonization and plant growth responses to inoculation using the commercial inoculum and to inoculation using native prairie soil.

Fungicide application in the field successfully reduced mycorrhizal colonization to <20% of colonization in non-treated control plots. Mycorrhizal colonization of roots in plots treated with the commercial inoculum did not differ from control plots. Suppression of mycorrhizal

fungi in the field experiment decreased the productivity of the dominant C_4 grasses (specifically *A. gerardii*), but did not increase abundance, cover, or abundance of seeded subordinate forb species within the two years of this study. Grass productivity and total aboveground net primary productivity were also reduced in the plots treated with commercial inoculum, contrary to our initial predictions. Species richness, evenness, and diversity did not significantly differ among treatments. However, the results highlight the importance of above- and belowground connections in restored tallgrass prairie, and indicate that temporarily suppressing AM fungi decreases cover and productivity of the dominant C_4 grasses (e.g. *A. gerardii*) in grass-dominated tallgrass prairie restorations. Because manipulating the mycorrhizal symbiosis can alter the dominance of the C_4 grasses in restored prairies, it may be possible to use fungicide to enhance the establishment and survival of subordinate forb species in restored grasslands, given a longer response time.

Key Words: *Andropogon gerardii*, arbuscular mycorrhizal fungi, fungicide, prairie restoration, productivity

Introduction

Arbuscular mycorrhizal (AM) fungi are ubiquitous in all plant communities. Approximately 80% of vascular plant species form symbiotic associations with AM fungi (Harley 1971). The beneficial relationship between AM fungi and host plants is well documented. Plants benefit by having increased nutrient uptake, increased drought tolerance, and protection from root pathogens (Perrin 1990; Fitter 1991; Marschner and Dell 1994; Ruiz-Lozano and Azcon 1995). In return, plants allocate as much as 26% of the carbon fixed by photosynthesis to the fungal symbionts (Miller et al. 2002). Additionally, AM fungi contribute to soil structure and aggregation (Bethlenfalvay et al. 1999; Rillig 2004a; Rillig and Mummey 2006; van der Heijden et al. 2006), promote decomposition (Hodge et al. 2001), mediate plant competition (Hartnett and Wilson 1999, 2002), alter water relations (Augé 2001), and enhance ecosystem carbon sink strength (Rillig 2004b). However, AM fungal colonization under certain conditions may provide no benefit to, or have negative effects on, the host plant (Bethlenfalvay et al. 1982; Fitter 1986; Francis and Read 1995; Grogan and Chapin 2000; Schwartz et al. 2006).

Besides being important in native and agricultural systems, mycorrhizal symbioses may also play a role in restoration. Landscapes available for restoration are typically highly disturbed in ways that negatively impact mycorrhizal fungi through reduced numbers and infectivity (Moorman and Reeves 1979; Jasper et al. 1989; Smith et al. 1998). Therefore, altering the soil communities may enhance successful plant recovery in grassland restorations. Studies have shown that disturbed habitats have reduced fungal propagules and lowered abundance and diversity of AM fungi (Miller 1979; Harris et al. 1993). Non-mycorrhizal plants are predominantly found in early successional environments (Reeves et al. 1979; Miller 1987); whereas late successional environments are often dominated by obligate or facultative AM plants (Allen and Allen 1990). Janos (1980) observed the pattern that as succession proceeds mycotrophic plant species replace less-AM-dependent plant species. AM fungi have been implicated as a mechanism to speed the rate of succession by acting as a biotic filter, recruiting into the community and providing a competitive advantage to late successional species which benefit the most from AM fungal colonization (Allen and Allen 1984). In addition, methods to promote plant recovery during restoration, such as fertilization, may alter the plant-fungal symbiosis from mutualism to parasitism (Johnson et al. 1997). Furthermore, if soil fertility is high at the restored site (e.g. restorations in agricultural soils), the symbiosis may become more parasitic since the plants do not need mycorrhizae to acquire the readily available soil nutrients (Anderson and Roberts 1993; Johnson et al. 1997).

Although the pivotal functions of AM fungi in natural systems are widely recognized and understood, there is less known about the role of mycorrhizae in restored grasslands and many questions remain regarding the possible use or manipulation of AM fungi to guide plant community recovery during restoration (Renker et al. 2004). Many studies have examined the occurrence of AM fungi in soils after restoration has occurred (Allen and Allen 1980; Corbett et al. 1996; Gould et al. 1996; Lovera and Cuenca 1996), while other studies have examined the potential for incorporating AM fungi into the restoration process. The latter studies found AM fungal inoculation to be beneficial during abandoned mine reclamation (Noyd et al. 1996; Thorne et al. 1998), revegetation of tropical soils degraded by an invasive grass (Cuenca et al. 1998), restoration of native grass species in a disturbed tallgrass prairie (Smith et al. 1998), and restoration of abandoned agricultural fields in semiarid grasslands (Richter and Stutz 2002).

During the initiation of a tallgrass prairie restoration the ratio of grass to forb species in the seed mixture is typically chosen based on a desired plant community composition and species diversity. However, the warm-season C_4 grasses often become more dominant than desired (Warkins and Howell 1983; Sperry 1994; Kindscher and Tieszen 1998), while establishment of subordinate grass and forb species is more difficult (Schramm 1976; Sperry 1983; Warkins and Howell 1983), leading to overall low species richness and diversity. If dominance by a few species of grasses is permitted to increase, the subordinate species will inevitably disappear (Howe 1999). In native tallgrass prairie, the dominant C_4 grasses have strong competitive effects on subordinate species (Collins 1987; Gibson and Hulbert 1987; Hartnett and Fay 1998), and manipulating factors that decrease their dominance may result in competitive release for the subordinate grass and forb species (Collins 1987; Hartnett et al. 1996; Howe 1999). Prairie forb species vary in their growth response to AM fungal colonization along a continuum from non-responsive, facultatively mycotrophic to highly responsive, obligately mycotrophic species (Wilson and Hartnett 1998) while the dominant C_4 grasses are strongly mycotrophic (Hartnett and Wilson 1999); therefore manipulating AM fungi during tallgrass prairie restoration may be useful in promoting establishment and growth of forb species in grass-dominated tallgrass prairie restorations.

This study was conducted to assess the role of AM fungi in structuring plant communities in restored grassland, and to evaluate the potential for manipulating community composition during tallgrass prairie restoration through the use of either commercial AM fungal inoculum or fungicide. A greenhouse study was set up to assess the inoculum potential and root colonization of plants grown in the commercial inoculum since this product was applied to the soil in the field experiment. Our specific objectives were to (1) assess the role of mycorrhizae in affecting plant specie composition (richness and diversity), productivity, cover, leaf tissue quality, as well as soil N and P availability in restored tallgrass prairie and (2) assess the effects of manipulation of mycorrhizae on seeded forb species that vary in degree of mycorrhizal dependence. Based on the strong differential responses of plant species to AM fungi in native grasslands (Wilson and Hartnett 1998), we hypothesized that changes in AM fungal abundance during tallgrass prairie restoration would lead to significant differences in plant species composition, cover, and diversity. More specifically, we hypothesized that: (1) since the dominant C_4 perennial grasses are highly dependent on AM fungal symbiosis (Hartnett and Wilson 1999), the fungicide

treatment would decrease the productivity and cover of the dominant grasses allowing for subordinate forb species to increase in cover and abundance; (2) mycorrhizal-dependent forb species would respond negatively to the fungicide treatments, similar to the dominant grasses; and (3) since facultative mycorrhizal forb species do not require the symbiosis, the cover or abundance of these forb species would increase through suppression of the dominant grasses and competitive release in the fungicide treatments. Given that AM propagules are generally present in grassland restorations (Corbett et al. 1996) as compared to highly disturbed sites targeted for restoration (e.g. reclaimed surface mines), we expected no significant differences in plant biomass, cover or growth in the commercial inoculum-treated plots relative to non-treated control plots. To test these hypotheses, forb species with varying growth responses to AM fungi, as determined in Wilson and Hartnett (1998), were over-seeded into an established grass-dominated restored prairie and changes in plant community composition and structure were measured in replicate plots that received a fungicide treatment to suppress AM fungi, were amended by a commercial inoculum, or were left untreated in a tallgrass prairie restoration established 8 years prior to the start of this study.

Methods

Field study site

Plots were located in a prairie restoration established in 1998 (see Baer et al. 2003) in a former lowland agricultural field at Konza Prairie Biological Station (KPBS), a 3487 ha tallgrass prairie preserve located in the Flint Hills region of Northeastern Kansas (39°5′N, 96°35′W). Mean annual precipitation at the site is 834 mm yr^{-1} (1891-2002) with high variability between years (coefficient of variation = 24%); approximately 635 mm falls as rain during the growing season (April through September) of each year (Sophocleous 1998). In the two years of this study (2006, 2007) total precipitation was 631, and 693 mm, of which 570, and 607 mm fell during the growing season (April through September) of each year, respectively. Even though 2006 growing season rainfall was ~90% of the long-term average, 301 mm occurred late in the growing season (August through September), and water stress was evident much of the growing season. The soil was a Reading silt loam (mesic Typic Argiudoll) formed by alluvial and colluvial deposits. Prior to restoration, the site had been cultivated for more than 50 years. Since the restoration, the area has become dominated by *Andropogon gerardii* Vitman and *Panicum*

virgatum L. (nomenclature follows USDA, NRCS Plants Database [2007]). Forb species were sparse throughout the restoration area at the start of this study. The entire restored field was burned frequently with prescribed spring fires.

Establishment of experimental field plots

In April 2006, following a prescribed spring burn, eighteen 3 × 3 m plots (with 2.5 to 3 m buffer strips between all plots) were delineated in a random complete block design (Figure 4-1). Three treatments, each replicated six times, were randomly assigned within a block. Within each block, AM fungi were suppressed in one plot by repeated application of a fungicide (Fungo®; thiophanate-methyl), one plot received a one-time application of a commercial AM fungal inoculum (Myco-bio-boost™), and one plot served as an untreated control. The fungicide and inoculum treatments were applied in the center 2 × 2 m square of each plot, providing a 1m buffer around each treatment. The fungicide treatment was applied as soil drench (7.5 L per plot) approximately every two weeks throughout the growing season (April through October) at a rate of 1.25 g m^{-2} (active ingredient). The control plots and inoculum plots received an equivalent volume of water (7.5 L) every two weeks. In the 2006, the commercial inoculum was broadcast applied and lightly raked into the soil according to manufacturer's instructions.

Plant community establishment

In April 1998, the dominant grasses (*Andropogon gerardii*, *Panicum virgatum*, *Schizachyrium scoparium*, and *Sorghastrum nutans*) were seeded with a grass drill throughout the research area at a rate of 10 pounds of live seed per acre (S. Baer, pers. comm.). Baer and others (1999, 2003) provide additional details regarding seed sources and restoration practices used at this site. In May 2006, 12 forb species were seeded into each plot at a rate of 100 seeds m^{-2} (Table 4-1). Prior to planting, all plots were lightly raked. The seeds were mixed with builder's sand and hand broadcast evenly over the plots. Forb species were selected to include a range of mycorrhizal responsiveness, based on prior studies that assessed growth responses of these species to AM colonization (Wilson and Hartnett 1998).

Due to poor establishment of newly seeded forbs in 2006, the same forb species were sown into research plots twice more in 2007 with the addition of *Salvia azurea* Michx. Ex Lam. This species was added because it established well during past seeding efforts in adjacent restoration plots, and it was used as an indicator species for potential establishment of seeds

sown in 2007. In April 2007, each species was sown at a rate of 400 seeds m^{-2}. Additional seeds were sown (at 400 seeds m^{-2}) after a 30-day exposure to 0˚C. Furthermore, forb seeds were tested for viability using tetrazolium chloride (1.0% 2, 3, 5-Triphenyl-2H-tetrazolium chloride), and for seed germination (Table 4-2).

Belowground sampling

Soil nutrient availability

Soils were sampled for available P (Bray test 1) and extractable inorganic N (NO_3^- and NH_4^+) prior to treatment in year one (only in control plots) and for all plots at the end of both growing seasons (2006 and 2007). Two soil cores (10 cm deep x 2 cm diameter) were collected and composited from each 2 × 2 m plot, crumbled by hand and sieved through 4-mm mesh to remove roots and rocks, and stored at 4˚C until being extracted. The commercial inoculum was also analyzed for available P and available inorganic N. The soil and inoculum analyses were conducted at the Soil Testing Lab at Kansas State University, Manhattan, KS.

Mycorrhizal colonization

To evaluate the effectiveness of the fungicide and the commercial inoculum, 2 soil cores (2 cm diameter × 10 cm deep) were removed from fungicide-treated, commercial inoculum-treated and control plots in late September in 2007. Roots were extracted from the soil, washed free of soil, and stained in trypan blue (Phillips and Hayman 1970). The roots were microscopically examined using a Petri dish scored in 1-cm squares to determine percent of roots colonized by mycorrhizal fungi (Daniels et al. 1981).

Aboveground sampling

Plant community indices

In both years, percent cover of each plant species was visually assessed in spring (late May- early June) and summer (August) for all plants rooted within a 0.25-m^2 quadrat in each plot. For each species, the maximum cover value from the spring and summer cover values was used for calculating plant species richness, diversity, and evenness. Species richness (S) was calculated as the number of plant species per 0.25-m^2 quadrat. Diversity was calculated for each plot using Shannon's diversity index, $H' = -\Sigma p_i \ln p_i$, where p_i represented the proportion of total cover contributed by species *i*. Shannon's diversity index was selected because it includes

proportional representation of species in a community and provides relatively even weighting to both richness and evenness (Barbour et al. 1999). Evenness was calculated using Pielou's index, $J = H'/H_{max}$, where H_{max} represented the natural log of S.

Productivity

At the end of each growing season (late August, early September), accumulated aboveground biomass was harvested from each plot in an area outside the species composition sampling quadrat ($n = 18$). Vegetation from one 0.25-m^2 area in each plot was clipped at ground level and sorted into the following categories: live grass, live forb, and surface plant litter. In 2007 only, biomass was sorted by grass and forb species. The surface litter material was minimal and was not classified as grass or forb matter; therefore, was excluded in calculating individual plot grass and forb productivity, but was included for calculating plot total ANPP. Biomass was oven-dried at 60°C for at least 48 hours, and each category was weighed separately then summed to estimate aboveground net primary productivity (ANPP), a measure of ecosystem function (Briggs and Knapp 1995). The biomass measurement represented all biomass for one year because the treatment plots were annually burned during the study period.

Leaf tissue quality

In 2007, 10 individual young leaves were collected at the end of the growing season in each plot from randomly selected individuals of *A. gerardii, P. virgatum,* and *S. nutans* in order to determine leaf tissue chemistry. Percent carbon, nitrogen and phosphorus were measured, and C:N, C:P, and N:P ratios were calculated as indices of leaf tissue quality. Percent total carbon and total nitrogen were determined by coupled combustion and gas chromatography on a CN Analyzer (Carlo Erba, Milan, Italy) from a subsample of finely ground leaf tissue, dried at 60˚C. Tissue phosphorus content was determined colorimetrically using ammonium molybdate and ascorbic acid as color reagents, following sulfuric acid/hydrogen peroxide digestion of plant tissue. Leaf tissue phosphorus content was analyzed by the Soil Testing Lab at Kansas State University, Manhattan, KS.

Greenhouse experiment

Soil preparation

Prairie soil, a Chase silty clay loam (mesic Aquic Argiudoll), was collected from Konza Prairie Biological Station, Manhattan, Kansas, and transported to a greenhouse at Kansas State University. A portion of the soil was steamed-pasteurized at 80˚C for 2 hours and allowed to cool for 72 hours with no measurable changes to soil chemistry (Wilson and Hartnett 1998) while the remaining soil was left untreated (nonsterile). Samples of field-collected soil were analyzed for mycorrhizal spore content and composition at Northern Arizona University by Nancy Collins Johnson. The nonsterile soil contained spores of 15 species of AM fungi, with *Glomus aggregatum*, *G. etunicatum*, *G. constrictum*, and *G. heterosporum* being the most common species present.

Plant preparation

Fungicide-free seeds of corn (*Zea mays*) were provided by Kansas State University Department of Agronomy and seeds of big bluestem (*Andropogon gerardii*) were provided by the Soil Conservation Service Plant Materials Center, Manhattan, KS. Seeds of *A. gerardii* were germinated in vermiculite. Fourteen days after emergence, 10 *A. gerardii* seedlings were individually transplanted into plastic pots (6 × 25) cm containing 400 g of steam-pasteurized soil (dry weight) soil. The appropriate treatment was applied by adding a layer consisting of 10 g of one of the following: 1) nonsterile soil, 2) steam-pasteurized soil amended with the commercial inoculum Myco-bio-boost^{TM}, 3) steamed-pasteurized soil amended with pasteurized commercial inoculum, or 4) steam-pasteurized soil not amended with inoculum. The treatment layer was followed by a final covering of 200 g sterile soil. Three *Zea mays* seeds were planted directly into pots established with the same soil treatments as described for *A. gerardii*. The commercial inoculum (pasteurized and nonsterile) was applied at the recommended rate provided by the manufacturer (50 lbs 1000 ft^{-2} or 0.0244 g cm^{-2}).

Experimental design and maintenance

Eighty pots were established in a split-plot design with the whole-plot factor being the four treatments (pots amended with: nonsterile soil, sterile soil, nonsterile commercial inoculum, or sterile commercial inoculum). Each whole-plot consisted of 20 pots of the same treatment.

The subplot factor was plant species (10 pots per species within each whole plot). Pots were arranged randomly on the greenhouse benches and re-randomized twice during the experiment to avoid any effects from bench locations. This eliminated the need for a block effect in the statistical analysis (Thomsen et al. 2006). Plants were watered daily and maintained in an 18-22˚C greenhouse for 8 weeks. After 8 weeks, plants were harvested and roots were washed free of soil. Plants were placed in a drying oven at 60˚C for 48 hours; then shoot, root, and total dry masses were recorded. Subsamples of dried roots were stained with trypan blue (Phillips and Hayman 1970) and percent root colonization was assessed microscopically using a Petri dish scored in 1-cm squares (Daniels et al. 1981).

Statistical analyses

Field study

Soil nutrient (N and P) availability, mycorrhizal colonization, vegetation responses (plant cover, leaf tissue quality, and productivity), and community indices (species richness, evenness, and diversity) were analyzed according to a random complete block design. Plant community responses to removal treatments were analyzed by year and across all years with repeated measure analysis. All data were analyzed using mixed-model analysis of variance (SAS Version 9.1; SAS Institute Inc. 2002-2003), with block as a random factor and treatment as a fixed factor. Denominator degrees of freedom were estimated using the Satterthwaite's method for all tests of fixed effects (treatment, time and treatment × time). All means comparisons were performed using the difference in least squares means procedure, $\alpha = 0.05$ (SAS 2002-2003).

Greenhouse study

Mycorrhizal responsiveness was calculated based on growth responses of *A. gerardii* and *Z. mays* plants inoculated with either native prairie soil containing AM fungal spores or with commercial inoculum (Myco-bio-boost^{TM}), relative to growth of non-inoculated plants grown in sterile soil. Percentage mycorrhizal responsiveness = [(dry mass mycorrhizal plant – dry mass nonmycorrhizal plant)/ dry mass mycorrhizal plant] × 100 (Wilson and Hartnett 1998). In the commercial inoculum treatment, the nonmycorrhizal plants used for these calculations were those grown in sterile soil amended with steamed-pasteurized Myco-bio-boost^{TM}. This was done to account for any nutrient addition associated with the use of the commercial inoculum.

Results

Field study

Percent root colonization

The bi-weekly fungicide treatments were effective in greatly reducing mycorrhizal root colonization ($p < 0.0001$), as compared to the inoculum-treated and control plots (Figure 4-2). However, the addition of commercial inoculum did not alter AM fungal colonization, compared to control plots.

Plant cover and productivity

Percent cover of grasses, forbs and total plant cover did not differ among treatments in either year, but 2007 had higher cover of grasses ($p = 0.0002$), forbs ($p = 0.0013$), and total plants compared ($p < 0.0001$) to 2006 (Table 4-3). End-of-season biomass values in 2006 did not differ among treatments, but in 2007 both fungicide-treated and commercial inoculum-treated plots produced less grass biomass ($p = 0.0086$) and total ANPP ($p = 0.0085$) compared to the control plots (Table 4-4). In 2007, biomass of each grass species was determined separately and *A. gerardii* biomass was reduced by both the fungicide and the commercial inoculum ($F = 7.71$, $p = 0.0094$; Figure 4-3). When comparing biomass production across years and treatments, both grass biomass ($p = 0.0065$) and total ANPP ($p = 0.0075$) were greater in 2007 control plots, as compared to other treatment-year combinations (Figure 4-4).

Community indices

Due to poor germination of forb species in both years, we were unable to assess the effects of the mycorrhizae manipulations on the growth of forb species that vary in degree of mycorrhizal dependence (Objective 2). In 2006, species richness was higher ($p = 0.0432$) in the control plots, compared to fungicide-treated and commercial inoculum-treated plots (however species richness are still quite low), but in 2007 there were no significant differences among treatments. Neither evenness nor species diversity were significantly affected by the treatments, but comparing between years, both species diversity ($p = 0.0076$) and species richness ($p = 0.0006$) were higher in 2007 (Table 4-5). When compared to native prairie on similar soil and

topography, all treatments were significantly lower in species richness, species evenness, and species diversity (Table 4-5).

Resource availability:

In 2006, neither the fungicide nor the commercial inoculum treatment significantly affected soil N or P availability (Table 4-6). In 2007, the fungicide treatment produced greater concentrations of KCl-extractable NH_4-N ($p = 0.0148$) and total inorganic N ($p = 0.0338$; Table 4-6).

Tissue analysis:

Tissue N content (%N) was greater in the fungicide treatment for *A. gerardii* ($p = 0.0031$), *S. nutans* ($p = 0.0084$), and *P. virgatum* ($p = 0.00162$; Table 4-7). Tissue C content (%C) was less in the fungicide treatment only for *S. nutans* ($p = 0.0072$) while there were no differences among treatments for *A. gerardii* and *P. virgatum.* Tissue P content (%P) did not vary significantly with treatment for any of the three species (Table 4-7). The C:N ratio was less in the fungicide treatment (Figure 4-5a) for *A. gerardii* ($F = 6.55$, $p = 0.0152$) and *S. nutans* ($F = 7.14$, $p = 0.0119$), but not for *P. virgatum* ($F = 1.90$, $p = 0.1992$). The N:P ratio was greater in the fungicide treatment (Figure 4-5b) for *S. nutans* ($F = 11.55$, $p = 0.0025$) and *P. virgatum* ($F = 11.39$, $p = 0.0026$), but not for *A. gerardii* ($F = 2.28$, $p = 0.1528$). The C:P ratio did not differ among treatments for *A. gerardii* ($F = 0.16$, $p = 0.8584$), *S. nutans* ($F = 0.18$, $p = 0.8408$), or for *P. virgatum* ($F = 1.17$, $p = 0.3500$).

Greenhouse study

Mycorrhizal responsiveness and root colonization

All plants grown in steam-pasteurized soil that was inoculated with native prairie soil survived and were colonized by mycorrhizal fungi (Table 4-8). However, plants grown in steam-pasteurized soil amended with the commercial inoculum experienced high mortality (only 6 of 10 *Z. mays* survived) and not all plants were colonized by mycorrhizal fungi (only 8 of 10 *A. gerardii* inoculated showed evidence of colonization, and % colonization was reduced by 80% relative to plants grown with native soil inoculum; Table 4-8). *Andropogon gerardii* seedlings grown in steam-pasteurized soil without mycorrhizal inoculation were not colonized, and failed

to grow. *Zea mays* seedlings were able to grow in the absence of native soil inoculum, but both non-inoculated and commercial-inoculated seedlings produced less biomass, compared to seedlings grown with native soil inoculum (Table 4-8). Percent mycorrhizal colonization of Z. mays seedlings was also greatly reduced with commercial inoculum, relative to seedlings grown with native soil inoculum.

Discussion

Originally, the restored site used for this study was planted only with warm-season grasses (S. Baer, pers. comm.), and it has become dominated by these grasses resulting in low species diversity. In order to increase abundance of subordinate species and overall plant species diversity, we hypothesized that it may be necessary to reduce the cover and productivity of the dominant grasses, specifically *A. gerardii*, in order to allow the subordinate species to establish and increase in relative cover, and in turn, enhance overall species diversity. Based on the role of plant mycorrhizal symbioses in native grassland communities (Hartnett and Wilson 1999), we hypothesized that the productivity and cover of the dominant, highly mycotrophic C_4 perennial grasses (e.g. *A. gerardii*) would decrease in the fungicide treatment, and with this temporary suppression of AM fungi, cover and abundance of subordinate forb species would increase.

The benefits of using mycorrhizal inoculation during restoration are well documented for studies conducted on depauperate sites, such as reclamation of taconite iron ore tailings (Noyd et al. 1996), rehabilitation of degraded tropical soils (Cuenca et al. 1998), and restoration of strip mines (Corbett et al. 1996). Besides using mycorrhizal inoculation in these degraded systems, mycorrhizae have also been shown to be beneficial in reestablishing vegetation, specifically the dominant grasses, early in grassland restorations (Richter and Stutz 2002; Smith et al. 1998). Even though early seral stages of prairie restoration are dependent on mycorrhizae, and adding AM fungi promotes the development of these young communities (Smith et al. 1998), few studies have examined the effect of AM fungi on older restored communities which have become dominated by strongly obligate mycotrophic grasses.

Hartnett and Wilson (2002) examined mycorrhizal regulation of plant competition and concluded that species diversity in communities dominated by obligate mycotrophs is greatly influenced by the symbiosis. The relative effects of mycorrhizal symbiosis on plant diversity may vary with degree of mycorrhizal dependency, which could explain the varying conclusions

documented. In some plant communities, mycorrhizae can increase plant species diversity through accelerated establishment of subordinates (Gange et al. 1993; Grime et al. 1987). While in other studies mycorrhizae have been shown to decrease species diversity via increased competitiveness of the dominants (Bergelson and Crawley 1988; Hartnett et al. 1993; Hartnett and Wilson 1999; Zobel and Moora 1995). In other cases, it appears that mycorrhizae have little effect on species richness (Smilauer and Smilauerova 2000). For studies in which AM fungi were reported to increase species diversity, the dominants were weakly mycotrophic. Therefore, the subordinates benefited from the symbiosis, presumably due to an increase in nutrient acquisition. In contrast, when the dominant species is strongly mycotrophic, AM fungi may decrease species diversity. Suppression of the symbiosis in these communities resulted in a reduction in dominance of the mycotrophic species (warm-season grasses), with a concomitant increase in facultatively mycotrophic species (e.g. forbs) due to competitive release. Tallgrass prairie communities fall into the latter group, with mycorrhizal responsiveness of the dominant C_4 grasses being the overriding factor driving species diversity in this system (Hartnett and Wilson 2002). Therefore, mycorrhizal suppression via fungicide would be expected to allow a competitive release with an increase in abundance of the subordinate grasses and forbs (Hartnett and Wilson 1999). This does not mean, however, that mycorrhizal fungi should always be suppressed during tallgrass prairie restoration. AM fungi have been shown to be required to establish the dominant grasses during restoration (Anderson et al. 1994; Cuenca et al. 1998; Hetrick et al 1989; Noyd et al. 1996), and can be beneficial to newly restored plant communities (Corbett et al. 1996; Richter and Stutz 2002; Smith et al. 1998). Furthermore, effects of the mycorrhizal symbiosis not only vary among species but plants within the same species also vary in their response to AM fungi at different life stages (Hartnett et al. 1994). Hartnett and others (1994) conducted a garden experiment to investigate the role of AM fungi in affecting seedling emergence, flowering and stem densities of several tallgrass prairie grasses and forbs. They used the fungicide benomyl to suppress AM fungi and found that the fungicide treatment had no significant effect on the warm-season grasses (*A. gerardii* and *P. virgatum*) seedling emergence, while in adults, the fungicide treatment reduced flowering of *A. gerardii* and *S. nutans* (Hartnett et al. 1994).

The site used for our study was dominated by *A. gerardii*, an obligate mycotroph. A similar outcome was also recorded by Sluis (2002) in a long-term prairie restoration where

species richness decreased with time while *A. gerardii* increased in frequency over time. An increase in dominance and associated reduction in species richness is detrimental to restoration efforts that seek to increase species richness and diversity, but could be beneficial to restoration activities aimed at increasing vegetation or erosion control. If a dominant species is an obligate mycotroph, then temporarily suppressing AM fungi may decrease cover and productivity of the dominant species.

Our first hypothesis was that the fungicide treatment would decrease the cover and abundance of the dominant grasses allowing the subordinate forb species to increase in cover and abundance. This hypothesis was partially supported with respect to the effect of the fungicide on the dominant grasses, but due to poor forb establishment we were unable to determine if the treatments affected the subordinate forb species. Mycorrhizal suppression by fungicide resulted in marked reduction in biomass and cover of *A. gerardii* in a restored prairie. The reduction in biomass was driven by the decline in abundance of *A. gerardii,* which is an obligate mycotrophic C_4 grass (Hartnett and Wilson 1999; Hetrick et al. 1990). Differences observed in biomass and cover between years were most likely driven by differences in precipitation with 2006 being dry, while 2007 was a relatively wet year. The reduction in biomass observed in 2007 within the commercial inoculum-treated plots was unexpected and contrary to our initial predictions. Bledsoe and others (1982) and Teste and others (2004) observed a similar undesirable direct consequence of commercial inoculum resulting in reduced production rather than increased production. This observation requires further research, as commercial inoculum may result in unexpected or negative consequences. Schwartz and others (2006) review the use and consequences of commercial inocula. Mycorrhizal fungi have been marketed for use in agriculture, horticulture, habitat restoration, bioremediation, and forestry with the promise of benefits to the soil and to the plants, but there could be beneficial or detrimental consequences of its use (Schwartz et al. 2006). The beneficial aspects include increased yields and survival, reduced fitness of invasive weeds, and increased soil carbon storage (Bethlenfalvay and Linderman 1992; Johnson 1998; Rillig 2004b), but the exact opposite results have also been found with the use of commercial inoculum with decreased yields and survival, improved fitness of weeds, and decreased soil carbon storage (Hendrix et al. 1992; Marler et al. 1999; Chapela et al. 2001). Based on these contrary results, Schwartz and others (2006) make a call for further

research on the use mycorrhizal fungal inoculum to better understand potential negative effects, as well as impacts on non-target areas through movement and spread.

Alterations in leaf and soil chemistry were observed following fungicide applications. For example, %N of leaf tissue was increased in *A. gerardii, S. nutans,* and *P. virgatum* by the fungicide treatment. The leaf C:N ratio was decreased by the fungicide treatment for *A. gerardii* and *S. nutans,* and the leaf N:P ratio increased for *S. nutans* and *P. virgatum* by the fungicide treatment. Furthermore, concentrations of extractable inorganic soil N increased with the fungicide applications. This increase in total extractable inorganic soil N may be a result of the degradation of AM fungi. Burke and others (2002) reported similar results with a fungicide treatment of benomyl resulting in decreased leaf C:N ratios, and speculated that the degradation of the AM fungi may have increased availability of soil N resulting in an increase of %N of leaf tissue.

Due to the ineffectiveness of the commercial inoculum and the poor forb growth observed across all treatments we were unable to assess how forb species of varying mycorrhizal dependence would respond to the treatments (Hypotheses 2 and 3). In 2006, a few forb species were present (*Vernonia baldwinii* Torr., *Asclepias verticillata* L., and *Symphyotrichum ericoides* (L.) G.L. Nesom), but overall forb cover and biomass were minimal compared to the cover and biomass of the grasses (Tables 4-4 and 4-5). In 2007, newly seeded forbs were beginning to establish across all treatments even though the total biomass of forbs decreased in the control plots. This outcome observed in the control plots was strongly influenced by an individual *V. baldwinii* plant in one plot that did not fare well in 2007. Percent forb cover increased in 2007 as well as species richness and diversity; therefore, treatment effects on forb species establishment and success may be observed in future years.

This research demonstrates the importance of considering the role of mycorrhizal symbiosis with warm-season, mycotrophic, C_4 grasses during restoration and management. We determined that the fungicide Fungo^{TM} was effective in reducing mycorrhizal colonization. Furthermore, our results indicated that fungicide application suppressed the dominant grass species; therefore, applying fungicide may be a useful tool during restoration and management of a restored prairie that has become grass-dominated by obligate mycotrophs, although we were unable to assess forb responses during the two-years of this study. We also determined that the commercial inoculum Myco-bio-boost^{TM} was ineffective at promoting root colonization by AM

fungi, and actually decreased aboveground net primary productivity in a restored tallgrass prairie. Myco-bio-boost^{TM} has been shown to be beneficial to plant growth in other greenhouse observations (J. Pizzo, pers. comm.), but this could be a fertilizer effect from application of the product, which contained 664 ppm-P, 3023 ppm-NH_4 and 24.2 ppm NO_3. In total, our results suggest that longer-term studies of the role of AM fungi in structuring restored prairie communities are warranted.

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Figures and Tables

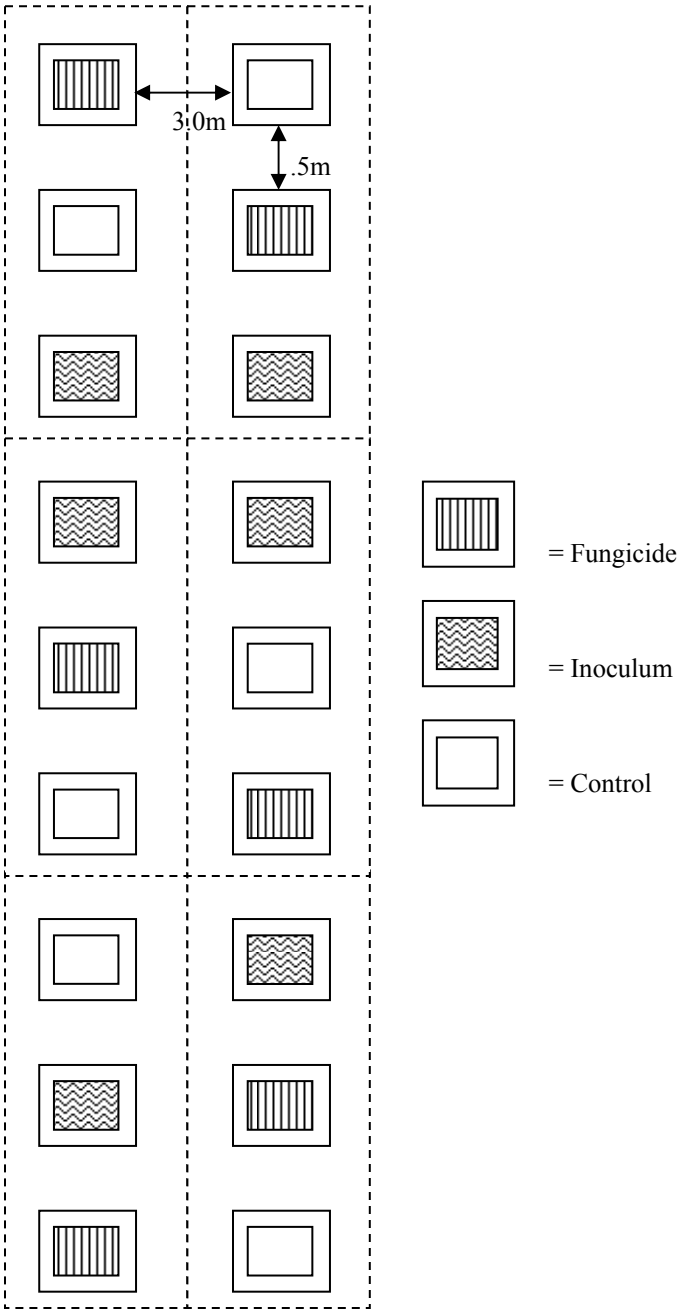


Figure 4-1. Plot layout of experiment on the role of mycorrhizae in restored prairie. The experimental design included control, fungicide, and commercial inoculum treatments initiated in 2006. Each plot treatment was randomly assigned within each block (dashed line) with 6 blocks total and 18 plots total. All plots were 3 × 3 m with treatments applied in the central 2 × 2 m area. The commercial inoculum treatment was applied only in 2006.

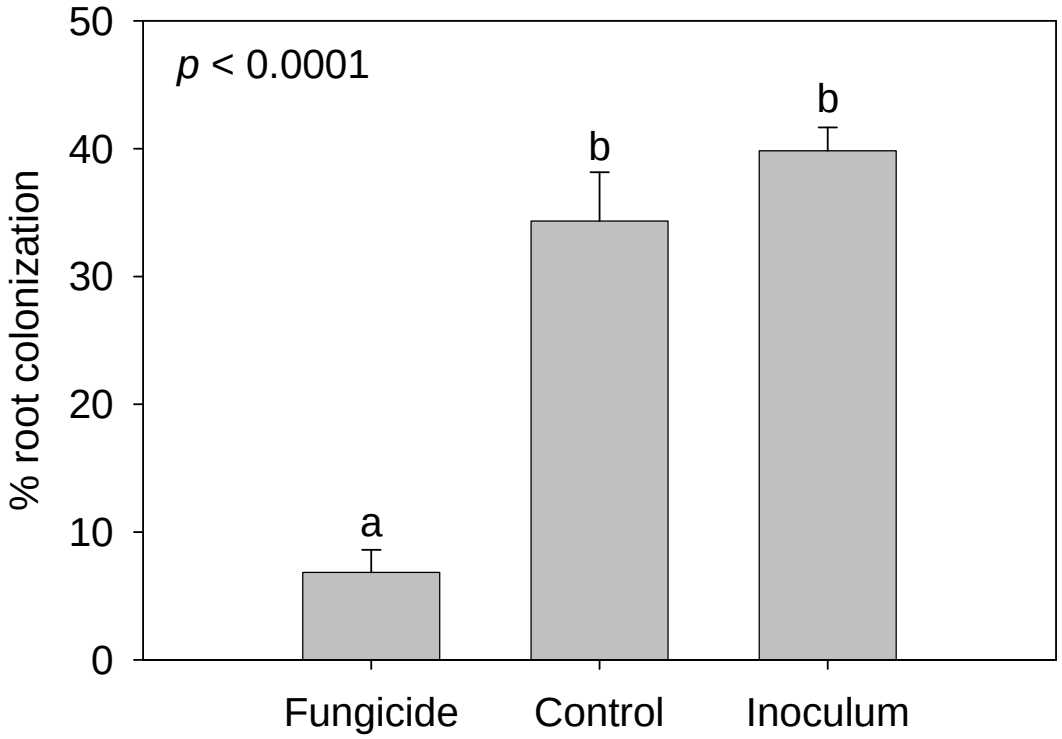


Figure 4-2. Mean (± SE) percent root colonization for the field study in 2007. Treatments included fungicide, control, and inoculum addition (n=6 for each treatment). Means accompanied by the same letter were not significantly different ($\alpha = 0.05$)

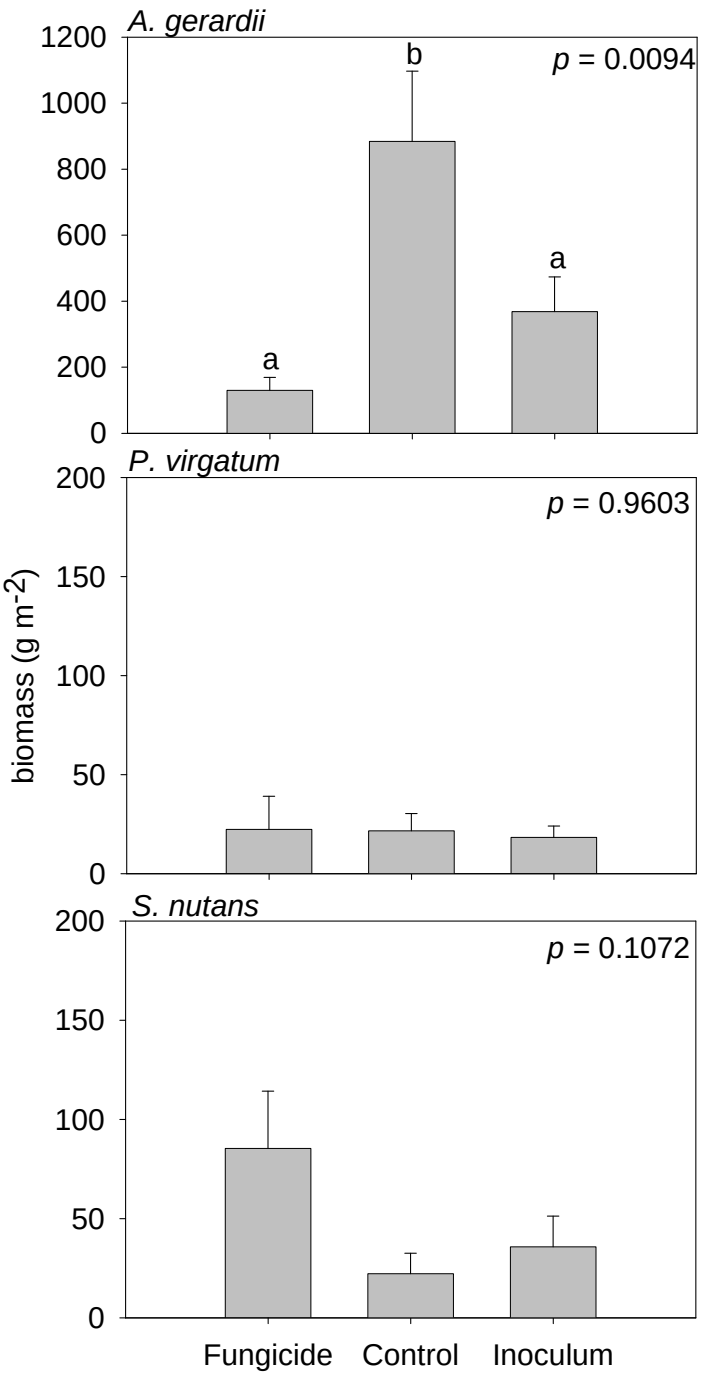
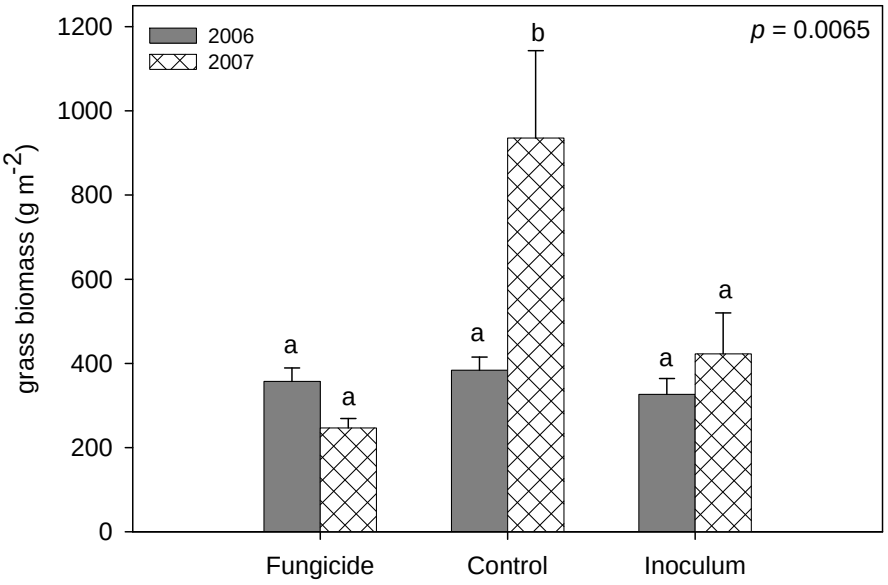


Figure 4-3. Mean (± SE) grass biomass (g m^{-2}) by species (*Andropogon gerardii*, *Panicum virgatum*, and *Sorghastrum nutans*) in 2007. Treatments included fungicide, control, and inoculum addition (n=6 for each treatment). Means accompanied by the same letter were not significantly different ($\alpha = 0.05$). Note different scales on y-axes.

A. Grass biomass 2006-2007 comparison



B. Total ANPP 2006-2007 comparison

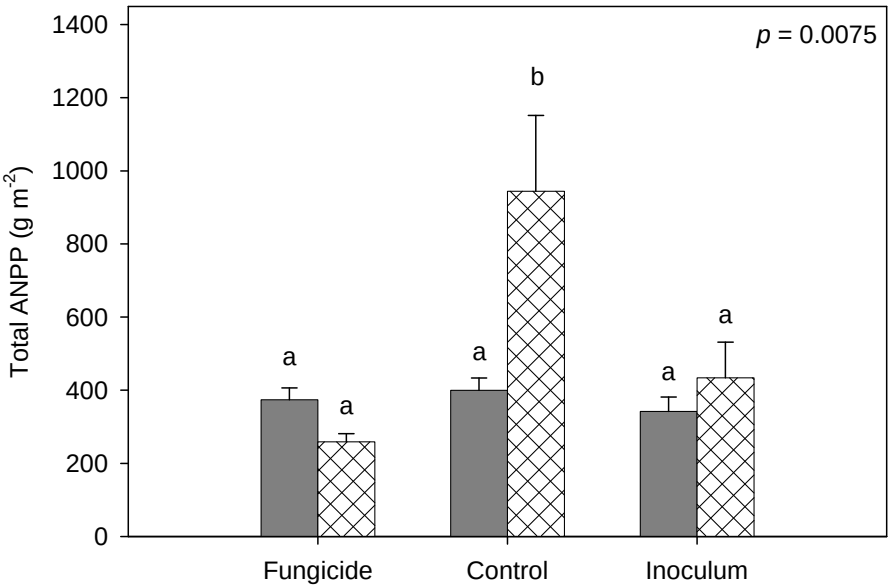


Figure 4-4. Mean (± SE) of biomass production (g m^{-2}) comparing 2006 with 2007 for grass biomass (A) and total ANPP (B). Treatments included fungicide, control, and inoculum addition (n=6 for each treatment). Means accompanied by the same letter were not significantly different ($\alpha = 0.05$), and *p*–values correspond to a significant interaction effect (treatment × time).

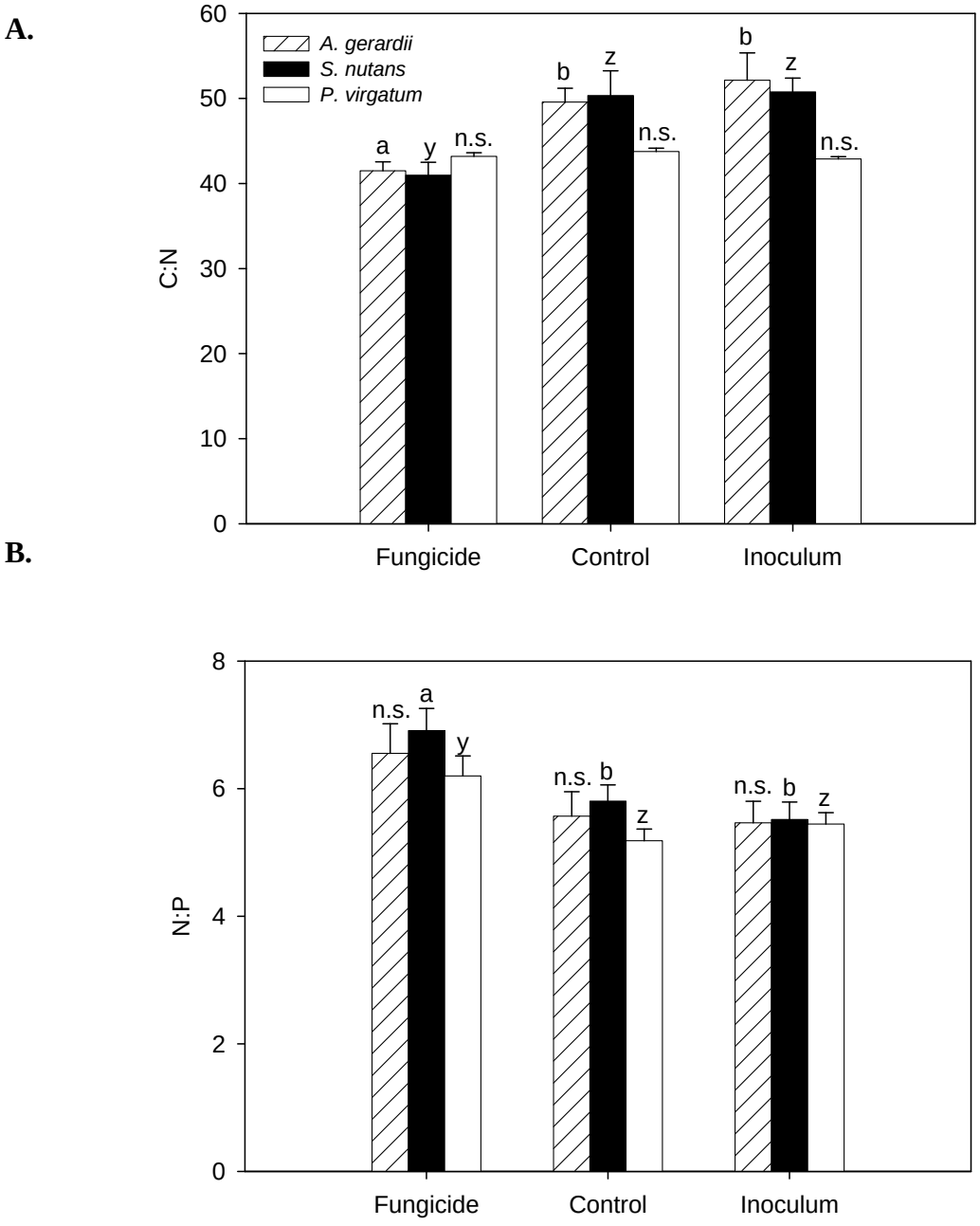


Figure 4-5. Mean (± SE) values for indices of leaf tissue chemistry, including A) C:N ratio and B) N:P ratio for samples collected in 2007 of *Andropogon gerardii, Sorghastrum nutans,* and *Panicum virgatum*. Treatments included fungicide, control, and inoculum addition (n=6 for each treatment). Means accompanied by the same letter or designated with "n.s." were not significantly different ($\alpha = 0.05$).

Table 4-1. Forb species added in 2006 (100 seeds m^{-2}) and in 2007 (two applications of 400 seeds m^{-2}) to a prairie restoration initiated in 1998. Forbs species were selected based on mycorrhizal responsiveness (% MR) reported by Wilson and Hartnett (1998). Nomenclature follows USDA, NRCS Plants Database (2007). Asterisk (*) depicts obligate mycotroph (Wilson and Hartnett 1998).

Perennial Forb Species	% MR	Supplier/Seed Source
Family Asteraceae		
Achillea millefolium L.	22.9	Missouri Wildflowers Nursery/ Missouri
Artemisia ludoviciana Nutt.	44.3	Prairie Moon Nursery/ N-Central Iowa
Brickellia eupatorioides (L.) Shinners	21.7	Prairie Moon Nursery/ Central Iowa
* *Helianthus maximiliani* Schrad.	92.9	Prairie Moon Nursery/ E. South Dakota
Liatris aspera Michx.	-0.4	Prairie Moon Nursery/ Central Iowa
* *Ratibida pinnata* (Vent.) Barnh.	96.0	Missouri Wildflowers Nursery/ Missouri
* *Symphyotrichum laeve* (L.) Á. Löve	96.7	Locally collected
Vernonia fasciculata Michx.	3.8	Prairie Moon Nursery/ Faribault Co., S MN
Family Fabaceae		
* *Baptisia australis* (L.) R. Br. ex Ait. f. var. *minor* (Lehm.) Fern.	85.2	Missouri Wildflowers Nursery/ Missouri
* *Lespedeza capitata* Michx.	98.0	Prairie Moon Nursery/ Missouri
Mimosa nuttallii (DC.) B.L. Turner	43.9	Missouri Wildflowers Nursery/ Missouri
Family Apocynaceae		
* *Asclepias tuberosa* L.	91.0	Missouri Wildflowers Nursery/ Missouri
Family Lamiaceae		
* *Salvia azurea* Michx. ex Lam. (2007 only)	87.8	Missouri Wildflowers Nursery/ Missouri

Table 4-2. Seed viability and germination test results for forbs sown into restoration in 2007. Ten seeds per species were used for each test. 1.0% tetrazolium chloride was used to test for seed viability. Percent germination was calculated after 14 days growth on moist filter paper placed in Petri dishes. Not all forb species were tested for seed viability due to minute size.

Species name	% Viable	% Germination
Achillea millefolium L.	N/A	0
Artemisia ludoviciana Nutt.	N/A	60
Asclepias tuberosa L.	70	60
Baptisia australis (L.) R. Br. ex Ait. f. var. *minor* (Lehm.) Fern.	50	0
Brickellia eupatorioides (L.) Shinners	20	100
Helianthus maximiliani Schrad.	100	80
Lespedeza capitata Michx.	80	90
Liatris aspera Michx.	60	40
Mimosa nuttallii (DC.) B.L. Turner	50	20
Ratibida pinnata (Vent.) Barnh	90	100
Salvia azurea Michx. ex Lam.	40	60
Symphyotrichum laeve (L.) Á. Löve	N/A	0
Vernonia fasciculata Michx.	0	10

Table 4-3. Means (± SE) of percent cover of grasses, forbs and total plants for the 2 years of this study. Treatments included fungicide, control, and inoculum addition (n=6 for each treatment). Within years, there were no significant differences among treatments. Significant main effects occurred for year, but not for treatment; differences among years (over all treatments) are indicated by letters y-z. Means accompanied by the same letter were not significantly different ($\alpha = 0.05$).

	Year 1 (2006)	Year 2 (2007)
A) % Cover of Grasses		
Fungicide	54.17 (9.44)	70.83 (16.67)
Control	48.33 (6.01)	83.33 (13.33)
Inoculum	48.33 (5.73)	80.00 (12.52)
Overall ($p = 0.0002$)	50.28 (1.94)^{y}	78.06 (3.74)^{z}
B) % Cover of Forbs		
Fungicide	2.17 (1.01)	3.67 (0.88)
Control	2.50 (0.76)	4.83 (1.72)
Inoculum	5.17 (4.00)	12.17 (6.18)
Overall ($p = 0.0013$)	3.28 (0.95)^{y}	6.89 (2.66)^{z}
C) % Cover of Total		
Fungicide	56.33 (9.75)	74.5 (15.99)
Control	50.83 (6.03)	88.17 (12.17)
Inoculum	53.50 (5.58)	92.17 (12.37)
Overall ($p < 0.0001$)	53.56 (1.59)^{y}	84.94 (5.35)^{z}

Table 4-4. Means (± SE) of biomass (g m^{-2}) for the 2 years of this study and across both years. Treatments included fungicide, control, and inoculum addition (n=6 for each treatment). Within each year, significant differences in treatment occurred, as indicated by superscript numbers 1-3. Significant main effects occurred for year and treatment; differences among treatments (over all years) are indicated by letters a-c and differences among years (over all treatments) are indicated by letters y-z. Means accompanied by the same letter were not significantly different ($\alpha = 0.05$).

	Year 1 (2006)	Year 2 (2007)	Across both years
A) Grass Biomass (g m^{-2})			($p = 0.0086$)
Fungicide	357.33 (31.8)	246.43 (22.67)[1]	301.88 (55.45)[a]
Control	386.93 (30.42)	935.23 (207.67)[2]	661.08 (274.15)[b]
Inoculum	326.70 (37.57)	422.67 (97.14)[1]	374.68 (47.98)[a]
Over all treatments ($p = 0.0272$)	356.99 (17.38)[y]	534.78 (206.59)[z]	
B) Forb Biomass (g m^{-2})			($p = 0.5971$)
Fungicide	0.08 (0.04)	0.87 (0.53)	0.48 (0.39)
Control	4.88 (4.69)	0.10 (0.24)	2.58 (2.31)
Inoculum	2.22 (1.44)	0.27 (0.65)	1.42 (0.80)
Over all treatments ($p = 0.2839$)	2.39 (1.39)	0.58 (0.17)	
C) Total ANPP (g m^{-2})			($p = 0.0085$)
Fungicide	373.82 (32.25)	258.68 (22.61)[1]	316.25 (57.57)[a]
Control	406.97 (35.20)	944.43 (207.05)[2]	675.70 (268.74)[b]
Inoculum	341.80 (39.74)	433.90 (97.36)[1]	387.85 (46.05)[a]
Over all treatments ($p = 0.0076$)	374.19 (18.81)[y]	545.62 (205.70)[z]	

Table 4-5. Means (± SE) of species richness (no. spp. $0.25m^{-2}$), evenness (J), and diversity (H') for 2 years and across both years. Treatments included fungicide, control, and inoculum addition (n=6 for each treatment). Within each year, significant differences in treatment are indicated by letters a-b. Significant main effects occurred for year, but not for treatment; differences among years (over all treatments) indicated by letters y-z. Means accompanied by the same letter were not significantly different ($\alpha = 0.05$).

	Year 1 (2006)	Year 2 (2007)
A) Species Richness		
Fungicide	3.00 (0.26)[a]	4.17 (0.31)
Control	4.17 (0.31)[b]	4.83 (0.60)
Inoculum	3.00 (0.37)[a]	4.67 (0.21)
Overall ($p = 0.0006$)	3.39 (0.39)[y]	4.56 (0.20)[z]
Native prairie	…	8.00 (0.93)
B) Species Evenness		
Fungicide	0.72 (0.11)	0.78 (0.03)
Control	0.76 (0.03)	0.72 (0.02)
Inoculum	0.78 (0.07)	0.75 (0.03)
Overall ($p = 0.9963$)	0.75 (0.02)	0.75 (0.02)
Native prairie	…	0.89 (0.02)
C) Species Diversity		
Fungicide	0.80 (0.13)	1.10 (0.05)
Control	1.07 (0.05)	1.12 (0.12)
Inoculum	0.81 (0.10)	1.15 (0.06)
Overall ($p = 0.0076$)	0.89 (0.09)[y]	1.12 (0.01)[z]
Native prairie	…	1.81 (0.11)

Table 4-6. Means (± SE) of inorganic soil N (μg N g soil^{-1}) and available P (μg P g soil^{-1}) for the 2 years of this study. Treatments included fungicide, control, and AM inoculum addition (n=6 for each treatment). Within each year, significant differences among treatments are indicated by letters a-b. Significant main effects occurred for year, but not for treatment; differences among years (over all treatments) are indicated by letters y-z. Means accompanied by the same letter were not significantly different (α = 0.05).

		Year 1 (2006)	Year 2 (2007)
Total inorganic N	Fungicide	15.57 (5.23)	10.85 (1.21)[a]
	Control	18.63 (5.97)	7.97 (0.89)[b]
	Inoculum	9.13 (2.44)	7.25 (0.88)[b]
	Type III, F, *P*	0.5362	0.0338
	Over all treatments (p = 0.0436)	14.44 (2.80)[y]	8.69 (1.10)[z]
NO_3-N	Fungicide	1.07 (0.08)	1.22 (0.19)
	Control	1.04 (0.11)	1.23 (0.20)
	Inoculum	1.13 (0.14)	1.02 (0.10)
	Type III, F, *P*	0.8391	0.6298
	Over all treatments (p = 0.5075)	1.08 (0.02)	1.16 (0.07)
NH_4-N	Fungicide	14.50 (5.25)	9.63 (1.06)[a]
	Control	17.59 (5.95)	6.74 (0.88)[b]
	Inoculum	8.01 (2.49)	6.23 (0.82)[b]
	Type III, F, *P*	0.3888	0.0148
	Over all treatments (p = 0.0430)	13.36 (2.82)[y]	7.53 (1.06)[z]
Phosphorus	Fungicide	24.43 (2.26)	19.25 (2.67)
	Control	27.33 (2.61)	26.65 (4.29)
	Inoculum	22.05 (1.39)	18.88 (1.40)
	Type III, F, *P*	0.1147	0.1216
	Over all treatments (p = 0.0509)	24.61 (1.53)	21.59 (2.53)

Table 4-7. Mean (± SE) concentrations (%) of leaf tissue N, C, and P for 3 grass species (*A. gerardii*, *S. nutans*, and *P. virgatum*) in year 2. Treatments included fungicide, control, and inoculum addition (n=6 for each treatment). Means accompanied by the same letter were not significantly different (α = 0.05).

	A. gerardii	*S. nutans*	*P. virgatum*
Tissue N (%)			
Fungicide	1.05 (0.03)[a]	1.06 (0.04)[a]	1.04 (0.05)[a]
Control	0.89 (0.03)[b]	0.89 (0.04)[b]	0.90 (0.04)[b]
Inoculum	0.84 (0.05)[b]	0.87 (0.03)[b]	0.88 (0.02)[b]
Type III, F, *P*	0.0031	0.0084	0.00162
Tissue C (%)			
Fungicide	43.50 (0.19)	43.11 (0.17)[a]	45.06 (0.45)
Control	43.76 (0.39)	44.13 (0.26)[b]	44.33 (0.46)
Inoculum	42.89 (0.27)	44.19 (0.19)[b]	44.64 (0.29)
Type III, F, *P*	0.0787	0.0072	0.2953
Tissue P (%)			
Fungicide	0.16 (0.011)	0.15 (0.008)	0.17 (0.008)
Control	0.16 (0.007)	0.15 (0.005)	0.17 (0.007)
Inoculum	0.15 (0.012)	0.16 (0.008)	0.16 (0.006)
Type III, F, *P*	0.8286	0.7834	0.5525

Table 4-8. Mean (± SE) dry weights of mycorrhizal and non-mycorrhizal plants, mycorrhizal responsiveness (MR) and mycorrhizal root colonization (RC) of *Andropogon gerardii* and *Zea mays* from the greenhouse study comparing native prairie soil inoculum and commercial (Myco-bio-boost™) inoculum.

		Mean Dry Weight			
Species	Inoculum Source	Myc.	Non-Myc.	MR (%)	RC (%)
A. gerardii	native soil	1.36 (0.078)	0.04 (0.005)	96.84	43.38 (2.10)
A. gerardii	Myco-bio-boost	0.04 (0.005)	0.04 (0.004)	4.55	9.00 (1.98)
Z. mays	native soil	3.07 (0.416)	1.55 (0.296)	48.50	52.44 (3.18)
Z. mays	Myco-bio-boost	1.70 (0.391)	1.44 (1.070)	15.49	8.33 (2.75)

MR = Mycorrhizal responsiveness (%) = [(mean dry mass mycorrhizal plant – mean dry mass nonmycorrhizal plant) / mean dry mass mycorrhizal plant] × 100 (Wilson and Hartnett 1998).

RC = Mean (± standard error) mycorrhizal root colonization (%).

CHAPTER 5 – SUMMARY AND CONCLUSIONS

Humans have the ability to induce environmental change while at the same time demanding goods and services from natural ecosystems. The human population will continue to grow, which will result in increase demand for ecosystem goods (i.e. food, fuel, timber, etc.). This added demand along with continued degradation and alteration will add more stress to already weakened ecosystems. With these added demands, conservation or simple maintenance of the current ecosystems will not be enough (Hilderbrand et al. 2005). Change in human consumption and demand will need to change, or a shift to creating, restoring, and enhancing ecosystems and their services will need to occur at a greater rate (Hilderbrand et al. 2005). A proactive, versus reactive, approach is necessary to protect, conserve, and restore the multi-functionality of ecosystems; otherwise, the remnants of these systems will continue down the degradation path without the hope of future rejuvenation.

With the loss and degradation of ecosystems, society expects and relies on "science to clean up the mess *and* make it look natural" (Hilderbrand et al. 2005). With this expectation restoration ecologists and ecological practitioners are developing strategies to restore degraded ecosystems. Restorations should not focus solely on restoring areas back to some "pristine" condition, since that condition may be unattainable, but some restorations should aim for the creation of novel ecosystems with ecosystem functioning (e.g. biodiversity and productivity) equivalent to pre-disturbance conditions.

The North American tallgrass prairie has been severely impacted by human activities since European settlement, with more than 96% of native prairies being lost due to conversion to agriculture, fragmentation, exotic species invasion, and fire suppression (Samson and Knopf 1994). With this loss, former and current tallgrass prairie ecosystems have experienced extensive alterations in ecosystem processes and community composition resulting in overall degradation. This degradation has led to decreased biodiversity and ecosystem services, and increased risk of exotic species invasion (Webb 1996; Hoekstra et al. 2005). The decline of tallgrass prairie ecosystems underscores the need to develop restoration methods to restore community composition and ecosystem services (Webb 1996).

The goal of most prairie restorations is to restore both dominant and subordinate species in proportions that reflect plant species diversity in native prairies, but the outcomes of many restoration attempts fall short of matching the diversity of their native counterparts (Thompson 1992; Howe 1994, 1995; Kindscher and Tieszen 1998). The initial planting phase of tallgrass prairie restorations typically starts with a grass-forb species mixture designed to provide a target level of diversity. However, the warm-season C_4 grasses often become dominant shortly after establishment (Warkins and Howell 1983; Sperry 1994; Kindscher and Tieszen 1998) while subordinate grass and forb species establishment is more difficult (Schramm 1976; Sperry 1983; Warkins and Howell 1983) leading to overall low richness and diversity. Determining the limitations to plant diversity in a restored tallgrass prairie is important for understanding the underlying ecological mechanisms involved, as well as for the design of more effective management practices.

In order to assess the potential for increasing forb abundance and species diversity in a grass-dominated tallgrass prairie restoration we examined three different methods for reducing the dominant grasses. These included (1) the direct physical/chemical removal of the dominant species, (2) the long-term manipulation of soil resources (soil depth and nutrient additions) and aboveground biomass removal via mowing, and (3) the manipulation of the mycorrhizal symbiosis with the dominant grasses. From the first investigation, we found that the physical removal of the dominant grasses, especially *Andropogon gerardii*, led to increased light availability, increased forb productivity and cover, as well as, increased species diversity and richness. The second investigation concluded that species diversity and richness were not affected by manipulations of soil nutrient availability, soil depth, or mowing; which could have been related to the high abundance of *A. gerardii* across all treatments. This suggests that manipulation of soil resources (N availability or soil depth) may not be sufficient to inhibit a highly dominant and well established C_4 grass species, at least within the time frame of this study. Longer-term management may be required to detect significant increases in forb abundance and cover, or further management may be required in order to enhance plant species diversity and richness in this grass-dominated prairie restoration. Lastly, the third investigation demonstrated the importance of considering the role of mycorrhizal symbiosis with warm-season, mycotrophic, C_4 grasses during restoration and management. Our results indicated that the fungicide application suppressed the dominant grass species; therefore, applying fungicide

may be a useful tool during restoration and management of a restored prairie that has become grass-dominated by obligate mycotrophs.

Overall, from these investigations we determined that restoring cover and diversity of forbs in a grass-dominated established restoration is difficult. In the short-term, a drastic reduction of the dominant grasses may be necessary to increase light availability allowing an opportunity for forb species to increase in cover and abundance. In addition, long-term reduction of N availability may be required to increase forb species if the restoration site is nutrient-rich (e.g. former agricultural field). Furthermore, during restoration the belowground biotic community must be considered, because these organisms (e.g. mycorrhizal fungi) are important in influencing the aboveground plant community in tallgrass prairie.

Continued long-term monitoring of these restoration plots will be required to assess if the forb species seeded in 2005 become established, and to determine if the conclusions provided from this study will hold true or if they will change over time. In addition, the restoration area is being invaded by crown vetch (*Coronilla* L.). In 2006 the patches of crown vetch were sprayed with herbicide, but continued management will be required to prevent further invasion of the species. Furthermore, this area has been more or less annually burned in the spring. This burn regime has been shown to increase the dominance of the warm-season grasses in tallgrass prairie while decreasing forb species (Howe 1994, 1995, 1999). Changing the burn regime and monitoring how the forb and grass species respond could be useful in further assessing the limitations of species diversity in established restored tallgrass prairie that has become grass-dominated. Future research needs that could be addressed in these plots could include bud bank responses in the dominant species removal treatment, investigation of soil microbial and mycorrhizal responses to the altered nutrient treatments, and lastly, termination of the fungicide treatment after the sown forbs become established to determine if they can compete when the dominant grasses regain their necessary mycorrhizal symbiotic associations. Understanding as much as we can about the competitive interactions occurring between subordinate and dominant species above- and belowground will only enhance our ability to design, implement, and manage effective sustainable tallgrass prairie restorations.

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